Lithium inhibits muscarinic-receptor-stimulated inositol tetrakisphosphate accumulation in rat cerebral cortex

Ian BATTY and Stefan R. NAHORSKI

Department of Pharmacology and Therapeutics, Medical Sciences Building, University of Leicester, University Road, Leicester LE1 7RH, U.K.

The effects of Li^+ on carbachol-stimulated phosphoinositide metabolism were examined in rat cerebral-cortex slices labelled with *myo*-[2- ^3H]inositol. The muscarinic agonist carbachol evoked an enhanced steady-state accumulation of [^3H]inositol monophosphate ([^3H]Ins P_1), [^3H]inositol bisphosphate ([^3H]Ins P_2), [^3H]inositol 1,3,4-trisphosphate ([^3H]Ins(1,3,4) P_3), [^3H]inositol 1,4,5-trisphosphate ([^3H]Ins(1,4,5) P_3) and [^3H]inositol tetrakisphosphate ([^3H]Ins P_4). Li^+ (5 mM), after a 10 min lag, severely attenuated carbachol-stimulated [^3H]Ins P_4 accumulation while simultaneously potentiating accumulation of both [^3H]Ins P_1 and [^3H]Ins P_2 and, at least initially, of [^3H]Ins(1,3,4) P_3 . These data are consistent with inhibition of inositol mono-, bis- and 1,3,4-tris-phosphate phosphatases to different degrees by Li^+ in brain, but are not considered to be completely accounted for in this way. Potential direct and indirect mechanisms of the inhibitory action of Li^+ on [^3H]Ins P_4 accumulation are considered. The present results stress the complex action of Li^+ on cerebral inositol metabolism and indicate that more complex mechanisms than are yet evident may regulate this process.

INTRODUCTION

There is now much evidence that several hormones and neurotransmitters enhance a receptor- and probably guanine-nucleotide-sensitive hydrolysis of $\text{PtdIns}P_2$ and that at least two of the products, Ins(1,4,5) P_3 and diacylglycerol, are important cellular second messengers [1,2]. More recent work indicates that Ins(1,4,5) P_3 can undergo either dephosphorylation to Ins(1,4) P_2 [3] or phosphorylation [4,5] to Ins(1,3,4,5) P_3 [6], and that the latter may also control cell activity by influencing Ca^{2+} entry across the plasma membrane [7].

Although at present there is a lack of pharmacological agents to perturb these systems, it has been known for some years that Li^+ can potentially inhibit the degradation of Ins P_1 [8], and this has been exploited in tissues such as brain to amplify Ins P_1 accumulation in the characterization and regulation of several neurotransmitter receptors and voltage-sensitive ion channels [9,10]. However, recent work suggests that Li^+ may also (albeit less potentially) inhibit the dephosphorylation of Ins(1,4) P_2 [3,11], and previous studies from our laboratory using rat cortex slices revealed a time- and concentration-related suppression of the carbachol-stimulated accumulation of radioactivity eluted in the '[^3H]Ins P_3 ' fraction from ion-exchange columns [12]. These results are now known to be complicated by the presence not only of two [^3H]Ins P_3 isomers but also of [^3H]Ins P_4 in this fraction [6]. We present evidence here that this inhibitory effect of Li^+ is predominantly on carbachol-stimulated [^3H]PtdIns P_4 accumulation in rat cerebral cortex.

MATERIALS AND METHODS

Cerebral-cortex slices (350 $\mu\text{m} \times 350 \mu\text{m}$) were prepared from male Sprague–Dawley rats and preincubated

for 60 min in Krebs–Henseleit buffer as previously described [12]. Samples (50 μl) of packed slices (1–2 mg of protein) were incubated in flat-bottomed vials with *myo*-[2- ^3H]inositol [NEN; 1–5 μCi (0.2–1.0 μM)/vial] for 60 min. After this period, Li^+ and the agonist carbachol were added, giving a final volume of 300 μl . It is important to note that carbachol stimulation was performed in the presence of [^3H]inositol to minimize major time-dependent changes in [^3H]inositol phosphate specific radioactivity. Incubations were terminated with 300 μl of either 7% (w/v) HClO_4 or 1.0 M-trichloroacetic acid, tissue was sedimented by centrifugation, and the supernatants were neutralized as described in [5] or [6] respectively. Inositol phosphates were separated on columns of Dowex 1 anion-exchange resin (200–400 mesh; formate form) or by gradient-elution h.p.l.c. as previously described [6].

RESULTS

Previous studies from our laboratory [12] have shown that Li^+ ions dose- and time-dependently potentiate muscarinic-receptor-mediated accumulations of [^3H]Ins P_1 and [^3H]Ins P_2 , but concomitantly decrease that of '[^3H]Ins P_3 ' in rat brain cortex. The '[^3H]Ins P_3 ' fraction originally studied has subsequently been resolved into three components, [^3H]Ins(1,4,5) P_3 , [^3H]Ins(1,3,4) P_3 and [^3H]Ins(1,3,4,5) P_3 [6]. Here we have extended our previous observations on the effects of Li^+ ions to each of these separate [^3H]Ins P fractions. As previously observed [12], Li^+ markedly potentiated carbachol-stimulated [^3H]Ins P_1 and [^3H]Ins P_2 accumulations, such that after 45 min these were respectively 8- and 2-fold greater than in the absence of this ion (Fig. 1). [^3H]Ins P_1 accumulation in the presence of Li^+ and carbachol was linear over the

Abbreviations used: PtdIns, phosphatidylinositol; PtdIns P , phosphatidylinositol 4-phosphate; PtdIns P_2 , phosphatidylinositol 4,5-bisphosphate; Ins P_1 , inositol monophosphate; Ins P_2 , inositol bisphosphate; Ins P_3 , inositol trisphosphate; Ins P_4 , inositol tetrakisphosphate.

Fig. 1. Effects of LiCl on the accumulation of [3 H]inositol phosphates stimulated by carbachol in cortex slices

Samples of cerebral-cortical slices (50 μ l; 1–2 mg of protein) were labelled with 1 μ Ci of [3 H]inositol for 30 min. Tissue was then further incubated for the indicated times in the presence and absence of 1 mM-carbachol and 5 mM-LiCl before extraction and subsequent analysis of the separate [3 H]inositol phosphates by Dowex anion-exchange chromatography. Results represent means $\pm$ S.E.M. for three experiments, and are expressed as radioactivity (c.p.m.) in each 3 H-labelled fraction in the absence (○) and presence (●) of agonist. Where present, LiCl was added 5 min before carbachol.

entire time course, whereas [3 H]InsP₂ rapidly achieved a maximum value, which was then maintained, suggesting that the hydrolysis of InsP₂ is less effectively inhibited at 5 mM-LiCl than is that of InsP₁ [12]. In the absence of Li⁺, the pattern of stimulated [3 H]InsP₂ accumulation was essentially the same as in its presence, whereas [3 H]InsP₁ only accumulated linearly for 10–15 min before reaching a new steady-state value (Fig. 1). When the antagonist atropine was added after first allowing carbachol-stimulated steady-state values to be achieved in the absence of Li⁺, the agonist-enhanced accumulation of each [3 H]InsP declined back close to control values (results not shown). Thus, as the antagonist can act only to block further muscarinic-receptor-mediated phosphoinositide hydrolysis, and not to reverse any preceding agonist-dependent increases in the specific labelling of the 3 H-labelled products, this demonstrates that little change in specific radioactivities occurs with the labelling protocol employed (see also [5]). This in turn shows that there is a continuous turnover of each [3 H]InsP in the presence of agonist, indicating that the muscarinic receptor is not desensitized under the conditions described. Basal accumulations of [3 H]InsP₁ and [3 H]InsP₂ were also somewhat potentiated by Li⁺, being 3- and 2-fold greater than in the absence of Li⁺ at 45 min.

The effects of Li⁺ on carbachol-stimulated [3 H]InsP₃ accumulation (Fig. 1) differ from those reported previously [12]. Muscarinic-receptor stimulation resulted in a rapid accumulation of [3 H]InsP₃ above control values, with close to maximum values being achieved within 5 min. However, whereas our previous studies demonstrated that, in the presence of Li⁺, more prolonged exposure to carbachol resulted in a severe decrease in [3 H]InsP₃, a similar effect was less apparent here. Thus, after 45 min in the presence of agonist and Li⁺, [3 H]InsP₃ accumulation was still about 80% of the value obtained at 10 min. In contrast, Li⁺ markedly decreased carbachol-stimulated [3 H]InsP₄ accumulation. Thus, initially carbachol resulted in a 20–50-fold increased accumulation of [3 H]InsP₄ in both the presence and the absence of Li⁺. However, although this maximal response was maintained for at least 45 min in the absence of the univalent ion, accumulation with Li⁺ declined sharply between 10 and 15 min, reaching a new steady state after 30–45 min. At this later time, carbachol-stimulated [3 H]InsP₄ accumulation was decreased by more than 65%. Thus it appears likely that the previous results [12] can be largely accounted for by a preferential decrease in [3 H]InsP₄ rather than Ins(1,4,5)P₃, as originally assumed. However, this interpretation is still complicated by the

presence of both [^3H]Ins(1,4,5) P_3 and [^3H]Ins(1,3,4) P_3 in the present [^3H]Ins P_3 fraction derived from cerebral cortex [6]. Thus it remained possible that Li^+ could potentiate [^3H]Ins(1,3,4) P_3 accumulation by inhibiting its hydrolysis, as reported by Burgess *et al.* [13] in pancreatic acinar cells, and this could offset a decreased accumulation of [^3H]Ins(1,4,5) P_3 , since its 5-phosphatase is apparently insensitive to Li^+ [14]. To establish the probable cause of the marked combined effects of Li^+ and carbachol on [^3H]Ins P_4 accumulation, the two trisphosphates were assayed by using gradient-elution h.p.l.c. Fig. 2 displays such data, and indicates that Li^+ at 5 mM potentiates [^3H]Ins(1,3,4) P_3 accumulation somewhat only over the first 15 min and that carbachol-stimulated [^3H]Ins(1,4,5) P_3 accumulation was not markedly influenced by this ion. These results are consistent with an inhibition of [^3H]Ins(1,3,4) P_3 hydrolysis by Li^+ , as previously suggested [13], perhaps

Fig. 2. Effects of LiCl on muscarinic-receptor-mediated [^3H]-Ins(1,3,4) P_3 and [^3H]Ins(1,4,5) P_3 accumulation in cerebral-cortical slices

Tissue incubations were performed as in the legend to Fig. 1, except that labelling was with 5 μCi of [^3H]inositol and continued for 60 min before drug treatments. Where present, LiCl was added at the same time as agonist. The [^3H]Ins P_3 species were analysed by h.p.l.c. Results are expressed as radioactivity accumulated in each [^3H]Ins P_3 fraction in response to 1 mM-carbachol above appropriate control values in the absence (○) or presence (■) of 5 mM-LiCl. Typical zero-time control values were 100–200 d.p.m. for [^3H]Ins(1,3,4) P_3 and 800–1000 d.p.m. for [^3H]Ins(1,4,5) P_3 . Results are from a single experiment, but were essentially identical on one further occasion.

followed by a decreased concentration of this [^3H]Ins P_3 consequent on the markedly attenuated accumulation of its immediate precursor, [^3H]Ins P_4 .

DISCUSSION

The present results demonstrate that low concentrations of Li^+ ions can severely disturb muscarinic-receptor-mediated inositol (poly)phosphate metabolism in brain. Firstly, at 5 mM, Li^+ ions potentiate [^3H]Ins(1,3,4) P_3 accumulation, but only initially. This action may explain our previous observation [12] that, at comparable times, dose/response curves to Li^+ of carbachol-stimulated total [^3H]Ins P_3 and [^3H]Ins P_4 accumulation are biphasic, with higher (> 1 mM) concentrations of Li^+ reversing the attenuating effects of lower concentrations (< 1 mM). Secondly, Li^+ ions have a major inhibitory effect on stimulated [^3H]Ins P_4 accumulation, but not on that of its precursor, [^3H]Ins(1,4,5) P_3 . The delayed onset of this action is also consistent with our previous studies [12], and argues against a direct inhibition or activation by Li^+ of Ins(1,4,5) P_3 3-kinase or Ins P_4 phosphatase respectively. An effect on the latter enzyme also seems unlikely, because a potentiated accumulation of [^3H]Ins(1,3,4) P_3 would then be predicted at later times, but was not observed.

The lag phase before the suppressant actions of Li^+ on stimulated [^3H]Ins P_4 accumulation became apparent suggests that, together, Li^+ and agonist either (a) cause depletion of a pool of an inositol-containing precursor or (b) induce accumulation of an inositol lipid metabolite, itself capable of exerting an inhibitory feedback influence. The former possibility is less likely, since this should ultimately be reflected in a decreased accumulation of each [^3H]Ins P and not specifically of [^3H]Ins P_4 . The second mechanism appears more probable, although its nature is unclear. A feedback mechanism initiated by [^3H]Ins P_1 and/or [^3H]Ins P_2 is unlikely, as our previous studies [12] have revealed suppressant effects of Li^+ on total [^3H]Ins P_3 and [^3H]Ins P_4 accumulation at concentrations of this ion which have only limited inhibitory actions on mono- and bis-phosphate hydrolysis. Alternatively, the attenuating effect of Li^+ on [^3H]Ins P_4 accumulation could be mediated indirectly via diacylglycerol or a related metabolite. This possibility is raised by reports that Li^+ ions potentiate diacylglycerol and CMP-phosphatidate accumulation in stimulated GH $_3$ cells [15] and parotid cells [16] respectively, whereas C-kinase-activating phorbol esters [17] can suppress receptor-stimulated Ins P accumulation [18,19] and accelerate Ins P_3 hydrolysis [20]. However, it should be recalled that inhibitory effects of Li^+ on total [^3H]PtdIns P_3 and [^3H]Ins P_4 accumulation are apparent at concentrations of this ion which would be unlikely to promote accumulation of diacylglycerol through impairment of phosphoinositide re-synthesis [12]. It should also be emphasized that, although the delayed onset of the Li^+ inhibition of stimulated [^3H]Ins P_4 accumulation strongly indicates mediation of this action of Li^+ via a secondary, unidentified, factor, this lag phase has only been demonstrated here at 5 mM-LiCl, and its full significance will only become clear when extensive time courses over a range of Li^+ concentrations have been completed.

Regardless of the mechanism by which Li^+ ions

decrease stimulated [^3H]Ins P_4 accumulation, it is evident from our present and previous results [12] that [^3H]Ins P_1 and [^3H]Ins P_2 concentrations are not correspondingly decreased, but rather are potentiated by Li^+ across the time course studied. This is consistent with the observation that under the current conditions Li^+ ions do not markedly influence [^3H]Ins(1,4,5) P_3 accumulation, although more extreme exposure to high concentrations of this ion, especially in the presence of agonist, might be expected to do so [21]. Thus it appears that, in the presence of Li^+ ions, carbachol-stimulated metabolism of [^3H]Ins(1,4,5) P_3 in cerebral cortex is diverted preferentially through direct dephosphorylation to [^3H]Ins P_2 by an as yet undefined mechanism.

Finally, comparison of the current results with those from other studies shows that brain inositol (poly)phosphate metabolism exhibits a number of features common to other tissues where Li^+ ions have similarly been demonstrated to potentiate stimulated [^3H]Ins P_1 , [^3H]Ins P_2 and [^3H]Ins(1,3,4) P_3 accumulation [13,22,23]. In contrast, the attenuating actions of Li^+ ions on stimulated [^3H]Ins P_4 accumulation shown here also suggest differences in brain inositol lipid/phosphate metabolism from that in other tissues [13,23]. Whether this latter effect of Li^+ ions is unique to brain remains to be established, although it is of interest that Hansen *et al.* [24] failed to observe a similar action in hepatocytes, although only early time points were examined. Clearly, a better understanding of the factors regulating inositol lipid and phosphate metabolism is required to explain the ways in which Li^+ ions exert their complex effects on this system in brain. However, as Ins(1,4,5) P_3 and Ins(1,3,4,5) P_4 may have second-messenger roles in brain, the decrease in the stimulated accumulation of the latter induced by Li^+ ions, and the expected consequent changes in metabolism of the former, could lead to altered cellular responses. The relationship, if any, of these effects to the therapeutic efficacy of Li^+ would seem worthy of further investigation.

This work is being supported by the Medical Research Council. We also thank Jenny Bell for manuscript preparation.

REFERENCES

- Berridge, M. J. & Irvine, R. (1984) *Nature (London)* **302**, 315–321
- Downes, C. P. & Michell, R. (1985) in *Molecular Mechanisms of Transmembrane Signalling* (Cohen, P. & Houslay, M., eds.), pp. 3–56, Elsevier, Amsterdam
- Storey, D. T., Shears, S. B., Kirk, C. J. & Michell, R. H. (1984) *Nature (London)* **312**, 374–376
- Irvine, R. F., Letcher, J., Heslop, J. P. & Berridge, M. J. (1986) *Nature (London)* **320**, 631–634
- Hawkins, P. T., Stephens, L. & Downes, C. P. (1986) *Biochem. J.* **238**, 507–516
- Nahorski, S. R. & Irvine, R. (1985) *Biochem. J.* **232**, 211–215
- Irvine, R. F. & Moor, R. M. (1986) *Biochem. J.* **240**, 917–920
- Hallcher, L. M. & Sherman, W. R. (1980) *J. Biol. Chem.* **255**, 10896–10901
- Nahorski, S. R., Kendall, D. A. & Batty, I. (1986) *Biochem. Pharmacol.* **35**, 2447–2453
- Downes, C. P. (1986) *Neurochem. Int.* **9**, 211–230
- Ackermann, K. E., Gish, B. G., Honchar, M. P. & Sherman, W. R. (1987) *Biochem. J.* **242**, 517–524
- Batty, I. & Nahorski, S. R. (1985) *J. Neurochem.* **45**, 1514–1521
- Burgess, G. M., McKinney, J. S., Irvine, R. F. & Putney, J. W. (1985) *Biochem. J.* **232**, 237–243
- Delvaux, A., Erneux, C., Moreau, C. & Dumont, J. E. (1987) *Biochem. J.* **242**, 193–198
- Drummond, A. H. & Raeburn, C. A. (1984) *Biochem. J.* **234**, 129–136
- Downes, C. P. & Stone, M. A. (1986) *Biochem. J.* **234**, 199–204
- Nishizuka, Y. (1984) *Nature (London)* **308**, 693–698
- Larbarca, R., Janowsky, A., Patel, J. & Paul, S. M. (1984) *Biochem. Biophys. Res. Commun.* **123**, 703–709
- Orellana, S. A., Solski, P. A. & Heller-Brown, J. (1985) *J. Biol. Chem.* **260**, 5236–5239
- Molina y Vedia, L. M. & Lapetina, E. G. (1986) *J. Biol. Chem.* **261**, 10493–10495
- Kendall, D. A. & Nahorski, S. R. (1987) *J. Pharmacol. Exp. Ther.* **241**, 1023–1027
- Berridge, M. J., Downes, C. P. & Hanley, M. R. (1982) *Biochem. J.* **206**, 587–595
- Thomas, A. P., Alexander, J. & Williamson, J. R. (1984) *J. Biol. Chem.* **259**, 5574–5584
- Hansen, C. A., Mah, S. & Williamson, J. R. (1986) *J. Biol. Chem.* **261**, 8100–8103

Received 26 May 1987/19 August 1987; accepted 26 August 1987