Beryllium competitively inhibits brain *myo*-inositol monophosphatase, but unlike lithium does not enhance agonist-induced inositol phosphate accumulation

W. Stephen FARACI,* Stevin H. ZORN,* Alice V. BAKKER, Elisa JACKSON, and Kara PRATT

Central Research Division, Pfizer Inc., Groton, CT 06340, U.S.A.

Despite limiting side-effects, lithium is the drug of choice for the treatment of bipolar depression. Its action may be due, in part, to its ability to dampen phosphatidylinositol turnover by inhibiting *myo*-inositol monophosphatase. Beryllium has been identified as a potent inhibitor of partially purified *myo*-inositol monophosphatase isolated from rat brain ($K_i = 150$ nM), bovine brain ($K_i = 35$ nM), and from the human neuroblastoma cell line SK-N-SH ($K_i = 85$ nM). It is over three orders of magnitude more potent than LiCl ($K_i = 0.5$ – 1.2 mM). Kinetic analysis reveals that beryllium is a competitive inhibitor of *myo*-inositol monophosphatase, in contrast with lithium which is an uncompetitive inhibitor. Inhibition of exogenous [^{3} H]inositol phosphate hydrolysis by beryllium ($IC_{50} = 250$ – 300 nM) was observed to the same maximal extent as that seen with lithium in

permeabilized SK-N-SH cells, reflecting inhibition of cellular *myo*-inositol monophosphatase. However, in contrast with that observed with lithium, agonist-induced accumulation of inositol phosphate was not observed with beryllium in permeabilized and non-permeabilized SK-N-SH cells and in rat brain slices. Similar results were obtained in permeabilized SK-N-SH cells when GTP- γ -S was used as an alternative stimulator of inositol phosphate accumulation. The disparity in the actions of beryllium and lithium suggest that either (1) selective inhibition of *myo*-inositol monophosphatase does not completely explain the action of lithium on the phosphatidylinositol cycle, or (2) that uncompetitive inhibition of *myo*-inositol monophosphatase is a necessary requirement to observe functional lithium mimetic activity.

INTRODUCTION

While lithium salts have been used in the treatment of bipolar affective disorder (manic-depressive illness), in recurrent unipolar depression and in some psychotic disorders other than mania, the site of activation underlying its therapeutic effectiveness remains obscure. Studies have shown that lithium is an uncompetitive inhibitor of *myo*-inositol monophosphatase (EC 3.1.3.25) [1]. Systemic administration of lithium leads to the depletion of free inositol levels and elevation of inositol monophosphates in rat brain tissue [2], thus the psychoactive effects of lithium may result in part from the depletion of inositol. The selectivity of this depletion in the central nervous system derives from the fact that neuronal cells have limited access to plasma inositol and must therefore depend on either *de novo* synthesis from glucose, which is also inhibited by lithium, or on recycling via the phosphoinositide pathway.

In the phosphatidylinositol second-messenger pathway, turnover of phosphatidylinositol occurs rapidly in response to extracellular stimuli [3]. As a consequence of polyphosphoinositide breakdown, two intracellular second messengers, diacylglycerol and inositol 1,4,5-trisphosphate, are liberated. It is widely accepted that inositol 1,4,5-trisphosphate modulates the mobilization of calcium from intracellular stores [4]. Hydrolysis of inositol 1,4,5-trisphosphate eventually leads to the liberation of free inositol, which is recycled via a series of metabolic steps to phosphatidylinositol in the membrane. *myo*-Inositol monophosphatase is the final enzyme in the phosphatidylinositol cycle which catalyses the hydrolysis of inositol 1-phosphate, inositol 3-phosphate and inositol 4-phosphate to inositol. This enzyme is

also utilized in the *de novo* synthesis of inositol from glucose 6-phosphate, via the intermediate *L*-*myo*-inositol 1-phosphate [5]. Thus, *myo*-inositol monophosphatase is an important enzyme in the phosphatidylinositol cycle since the generation of free inositol in brain tissue is dependent upon its action.

Recently, a number of groups have reported studies on the purification and characterization of the *myo*-inositol monophosphatase from bovine brain [1,6,7] and rat brain [8], as well as a detailed kinetic analysis of enzyme catalysis [9]. Kinetic analysis shows that lithium uncompetitively inhibits *myo*-inositol monophosphatase ($K_i = 0.8$ mM, [1]). Lithium also inhibits *myo*-inositol monophosphatase *in vivo* in the therapeutic range (0.5–1.5 mM), which may explain the decrease in inositol levels and the increase in inositol 1-phosphate levels in brain after systemic administration of LiCl to rats [10,11]. These findings have contributed to the hypothesis that *myo*-inositol monophosphatase is a likely target for lithium in the treatment of bipolar affective disorder [12,13], although other mechanisms have been proposed [14–16].

Thus, specific inhibitors of *myo*-inositol monophosphatase would be useful in determining whether enzyme inhibition is the principle mode of action of lithium. The present study was undertaken to characterize and examine the properties of beryllium as an inhibitor of *myo*-inositol monophosphatase. Although it is known that beryllium weakly inhibits alkaline phosphatase ($IC_{50} = 1$ μ M; [17,18]), there have been no reports on the effects of this ion on *myo*-inositol monophosphatase catalysis. Our results indicate that beryllium is a potent, competitive inhibitor of *myo*-inositol monophosphatase, though it does not enhance agonist-induced inositol phosphate accumu-

Abbreviations used: INT, Iodonitrotetrazolium Violet; DMEM, Dulbecco's modified Eagle's medium; GTP- γ -S, guanosine 5'-[γ -thio]triphosphate; GDP- β -S, guanosine 5'-[β -thio]diphosphate.

* To whom correspondence should be addressed.

lation in intact or permeabilized systems. These results suggest that either selective inhibition of *myo*-inositol monophosphatase does not completely explain the action of lithium on the phosphatidylinositol cycle, or that uncompetitive inhibition of *myo*-inositol monophosphatase is a necessary requirement to observe functional lithium-mimetic activity.

EXPERIMENTAL

Materials

The following materials were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A.: Trizma base, MgCl_2 , EGTA, KCl, Malachite Green, molybdic acid (ammonium salt), inosine, Iodonitrotetrazolium Violet (INT), GTP- γ -S, GDP- β -S, and DL-*myo*-inositol 1-phosphate. Nucleoside phosphorylase (5 mg/ml; 20 units/mg) and xanthine oxidase (20 mg/ml; 1 unit/mg) were purchased from Boehringer-Mannheim Biochemicals. *myo*-[2- ^3H]Inositol (12.3 Ci/mmol) was purchased from New England Nuclear. [^3H]Inositol 1-phosphate (9.2 Ci/mmol; 10 $\mu\text{Ci/ml}$), [^3H]inositol 4-phosphate (2.5 Ci/mmol; 10 $\mu\text{Ci/ml}$), [^3H]inositol 1,3,4-trisphosphate (5.7 Ci/mmol; 10 $\mu\text{Ci/ml}$) and [^3H]inositol 1,4,5-trisphosphate (17 Ci/mmol; 10 $\mu\text{Ci/ml}$) were purchased from NEN Research Products.

Partial purification of *myo*-inositol monophosphatase

The enzyme activity was isolated according to published procedures [6,7]. Fresh bovine brain was obtained from the local slaughterhouse and placed immediately on ice. Half a bovine brain was chopped into small pieces and homogenized using a Polytron homogenizer in 25 ml of 50 mM Tris buffer (pH 8.0) containing 0.1 mM EGTA. After centrifugation (27000 g), the enzyme was precipitated with 40–60% (w/v) ammonium sulphate and the precipitate redissolved in the above buffer. The enzyme was dialysed against the same buffer, applied to a DEAE anion-exchange column (2.5 cm $\times$ 15 cm) and eluted with a 0–0.5 M KCl gradient. Active fractions were concentrated, dialysed against 50 mM Tris buffer, pH 8.0, containing 0.1 mM EGTA, applied to an Ultrogel AcA 34 column (IBF Biotechnics) (2.5 cm $\times$ 85 cm) and eluted using the same buffer. Fractions containing enzyme were concentrated and stored in 40% (w/v) glycerol at -80°C . *myo*-Inositol monophosphatase from a human neuroblastoma cell line (SK-N-SH) and rat brain was partially purified as described above, except that the enzyme was not applied to the Ultrogel column.

Analytical methods

myo-Inositol monophosphatase activity was measured in 1.0 ml of 0.1 M Tris buffer (pH 7.4; 25°C) containing 2 mM MgCl_2 , 0.25 M KCl and 1.0 mM DL-inositol 1-phosphate according to the method described in [7]. In this assay, free phosphate reacts with inosine to give hypoxanthine and ribose 1-phosphate (catalysed by nucleoside phosphorylase). Hypoxanthine is oxidized to uric acid by xanthine oxidase concomitant with the reduction of INT to give a red formazan, which is detected spectrophotometrically ($\lambda_{\text{max}} = 546 \text{ nm}$). Neither nucleoside phosphorylase nor xanthine oxidase are affected by beryllium (results not shown). Another assay method utilized was the determination of free phosphate produced from inositol 1-phosphate hydrolysis. An Eppendorf tube containing 0.1 M Tris buffer (pH 7.4; 25°C), 2 mM MgCl_2 , 0.25 M KCl and DL-

inositol 1-phosphate was incubated with enzyme and, after a period of time, the reaction was quenched and free phosphate levels were measured using the Malachite Green spectrophotometric assay [19]. To determine the type of inhibition, substrate (0.1–1.5 mM) was incubated in buffer with BeCO_3 (0–0.4 μM) or LiCl (0–5 mM) to which enzyme was subsequently added. Collective constants (V_{max} , K_m , K_i) were estimated with the programs HYPER, COMP, and UNCOMP [20].

Cell-culture conditions

Human neuroblastoma SK-N-SH cells (passages 38–60) were grown in tissue culture flasks (175 cm^2 flasks) in 50 ml of Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) fetal calf serum. Cells were grown as described by Fisher and Snider [21].

Measurement of endogenous inositol phosphate accumulation in permeabilized cells

Confluent cultures of SK-N-SH cells were prelabelled for 2 days in 25 ml of DMEM/10% (v/v) fetal calf serum containing 16 $\mu\text{Ci/ml}$ [^3H]inositol, in an atmosphere of 95% air/5% CO_2 at 37°C . Cells were detached using 2 mM EDTA in Dulbecco's PBS without calcium and magnesium, washed (using the same buffer) and resuspended in KGEH buffer (139 mM potassium glutamate, 2 mM ATP, 4 mM MgCl_2 , 10 mM EGTA and 30 mM sodium Hepes buffer, pH 7.4). Cells were permeabilized with 10 μM digitonin according to the method of Fisher et al. [22] to ensure the entry of exogenous agents into the cells, and assayed for the accumulation of radiolabelled inositol phosphates induced by carbachol or GTP- γ -S in the presence of BeCl_2 (0.01–100 μM), LiCl (10 mM), and GTP- β -S (2 mM) according to Fisher and Snider [21]. Cells were incubated with carbachol (500 μM) or GTP- γ -S (100 μM) for 30 min, upon which the reaction was terminated by the addition of 1.5 ml of chloroform/methanol (1:2, v/v). Incubation for 30 min insures that inositol polyphosphates generated as a result of receptor stimulation are nearly fully metabolized (80%) to inositol monophosphates in the presence of lithium [21]. Isolation of inositol phosphates was achieved using ion-exchange chromatography [21].

Measurement of exogenous inositol phosphate hydrolysis in permeabilized cells

Hydrolysis of exogenously added radiolabelled inositol phosphate to free inositol was examined in permeabilized SK-N-SH cells. Two flasks of SK-N-SH cells were grown to confluence and permeabilized as described above. The cells were gently resuspended in 10 ml of KGEH buffer and aliquots (0.5 ml) were distributed into 1 ml Eppendorf tubes. To each tube was added 5 μl of a [^3H]inositol phosphate standard (final concentration of 10–40 nM) and 10 μl of beryllium salt [BeCl_2 , BeCO_3 , BeSO_4 , beryllium oxalate, and $\text{Be}_3(\text{PO}_4)_2$] at a concentration of 0–10 μM . A control tube containing [^3H]inositol phosphate and 10 mM LiCl was also present in each experiment. The tubes were placed in a 37°C water-bath for 2 h to allow enough time for [^3H]inositol phosphate to be almost completely metabolized to inositol. The reaction was terminated by addition of 1.5 ml of chloroform/methanol (1:2, v/v). Separation of [^3H]inositol from phospholipids and inositol phosphates was achieved by adding 2 ml of water and 1 ml of chloroform to each tube, transferring the aqueous layer to a tube containing 0.5 ml of Dowex AG 1-X8, vortex mixing for 10 min, and assaying the resulting aqueous layer for [^3H]inositol content by liquid scintillation spectrometry.

Measurement of inositol phosphate accumulation in rat brain slices

[³H]Inositol phosphate accumulation in rat brain slices was examined using a modification [23] of the method originally described by Brown et al. [24]. In brief, rat brain cerebral cortical slices (0.35 mm × 0.35 mm) were prepared using a McIlwain tissue chopper, and then incubated for 45 min at 37 °C in a modified Krebs–Ringer buffer (NaCl, 118 mM; KCl, 5 mM; CaCl₂, 1.3 mM; MgSO₄, 1.2 mM; NaHCO₃, 25 mM; and dextrose, 11.1 mM) oxygenated with 95 % O₂/5 % CO₂. During this time the buffer was changed three times at 15 min intervals. The slices were then pre-incubated for 15 min with either LiCl or BeCl₂ and then prelabelled for 30 min at 37 °C with *myo*-[³H]-inositol (1 μCi/tube) in the presence or absence of either LiCl or BeCl₂. After the prelabelling period, slices were exposed to the various test agents for 45 min under the same experimental conditions. The reaction was terminated by the addition of a chloroform/methanol (1:2, v/v) solution, and the water-soluble [³H]inositol phosphate was isolated by a batch technique employing a Dowex AG 1-X8 anion-exchange resin [24].

RESULTS

To study the kinetics of inhibition of *myo*-inositol monophosphatase by beryllium, the hydrolysis of inositol 1-phosphate by partially purified enzyme preparations was examined as described in the Experimental section. Inhibition of enzyme derived from bovine brain, rat brain, and a human neuroblastoma cell line (SK-N-SH) with beryllium yielded inhibition constants (*K_i*) of 35 ± 5, 150 ± 40, and 85 ± 15 nM respectively (Table 1). Analysis of the inhibition curves generated with bovine brain and rat brain enzymes indicates that beryllium competitively inhibits the hydrolysis of inositol 1-phosphate (Figures 1a and 1b). A number of beryllium salts, including Be₃(PO₄)₂, BeCO₃, BeSO₄, and BeC₂O₄ were equipotent with BeCl₂ as inhibitors of the bovine enzyme, indicating that bivalent beryllium ion is the inhibiting species (Table 2). These results are in contrast with those observed for LiCl, which was found to be an uncompetitive inhibitor of bovine and rat brain enzymes by Hallcher and Sherman [1] and confirmed in the present study (Table 1 and Figure 1c). Moreover, lithium is approx. 3–4 orders of magnitude less potent than beryllium as an inhibitor of *myo*-inositol monophosphatase (Table 1).

To study the inhibition of endogenous *myo*-inositol monophosphatase, SK-N-SH cells were permeabilized with digitonin and preloaded with various [³H]inositol phosphates. The liberation of [³H]inositol by inositol phosphate hydrolysis was

Figure 1 Inhibition of partially purified bovine and rat brain inositol monophosphatase by BeCO₃ and LiCl

Hydrolysis of *myo*-inositol 1-phosphate under initial velocity conditions was measured as described in the Experimental section. (a) Enzyme isolated from bovine brain; BeCO₃ concentration is 0 (●), 25 (○), 50 (×), and 100 nM (■). (b) Enzyme isolated from rat brain; BeCO₃ concentration is 0 (●), 250 (○), 500 (×), and 750 nM (■). (c) Enzyme isolated from bovine brain; LiCl concentrations are 0 (●), 1 (○), 2 (×), and 5 mM (■). The theoretical lines depicted are from a fit of all the data using the program COMP and UNCOMP [20].

Table 1 *In vitro* inhibition of *myo*-inositol monophosphatase activity isolated from rat, human, and bovine brain tissues

K_i values were derived from concentration–response curves and represent the concentration of beryllium ± S.E.M. that inhibits the hydrolysis of inositol 1-phosphate (100 μM, which is ≪ *K_m*) by 50%. Initial rates were measured as a function of time.

Enzyme source	<i>K_i</i> (μM)		<i>K_i</i> LiCl/ <i>K_i</i> BeCl ₂ ratio
	LiCl	BeCl ₂	
SK-N-SH	450 ± 50	0.085 ± 0.015	5300
Bovine brain	1100 ± 100	0.035 ± 0.010	31 000
Rat brain	550 ± 50	0.150 ± 0.040	3700

Table 2 *In vitro* potency of various beryllium salts as inhibitors of bovine brain *myo*-inositol monophosphatase activity

K_i values were derived from concentration–response curves and represent the concentration of beryllium salt ± S.E.M. that inhibits the hydrolysis of inositol 1-phosphate (100 μM, which is ≪ *K_m*) by 50%. Initial rates were measured as a function of time.

Compound	<i>K_i</i> (nM)
BeCl ₂	35 ± 10
BeCO ₃	35 ± 10
BeSO ₄	35 ± 10
BeC ₂ O ₄	35 ± 10

Figure 2 Inhibition of exogenously added $[^3\text{H}]$ inositol 1-phosphate hydrolysis by BeCl_2 in digitonin-permeabilized SK-N-SH human neuroblastoma cells

Each point represents the mean $\pm$ S.E.M. of triplicate determinations. Cells were exposed to the ^3H -labelled substrate for 2 h in the presence and absence of inhibitor.

Table 3 Inhibition of hydrolysis of exogenously added inositol monophosphates in digitonin permeabilized SK-N-SH human neuroblastoma cells

Digitonin-permeabilized cells were exposed to $[^3\text{H}]$ inositol phosphates for 2 h in the presence and absence of inhibitor. The liberated $[^3\text{H}]$ inositol represents the mean $\pm$ S.E.M. of four data points and was measured as described in the Experimental section.

Treatment	$[^3\text{H}]$ inositol liberated (d.p.m.)	
	$[^3\text{H}]$ inositol 1-phosphate	$[^3\text{H}]$ inositol 4-phosphate
Basal	25950 $\pm$ 501	17324 $\pm$ 1030
Lithium (10 mM)	5041 $\pm$ 80	2868 $\pm$ 123
BeCl_2 (10 μM)	3500 $\pm$ 736	1902 $\pm$ 226

assessed in the presence and absence of beryllium and lithium. As shown in Figure 2 and Table 3, BeCl_2 inhibited the hydrolysis of exogenously added inositol 1-phosphate (10–40 nM) in a dose-dependent manner with an IC_{50} of 300 ± 40 nM. Moreover, beryllium also inhibited the hydrolysis of exogenously added $[^3\text{H}]$ inositol 4-phosphate (10–40 nM) with an IC_{50} value of 250 ± 50 (results not shown).

To determine whether beryllium possesses lithium-mimetic activity, permeabilized SK-N-SH cells and rat brain cerebral cortical slices were prelabelled with *myo*- $[^3\text{H}]$ inositol, exposed to the muscarinic agonist carbachol in the presence and absence of beryllium and lithium, and the accumulation of $[^3\text{H}]$ inositol phosphate was measured. In permeabilized SK-N-SH cells (Figure 3a) and in rat brain cerebral cortical slices (Figure 3b), muscarinic agonist induced phosphatidylinositol turnover, as indicated by the increase in the accumulation of $[^3\text{H}]$ inositol phosphate was observed. This response occurred at a concentration of lithium that was 20-fold higher than its K_i value. In contrast with that observed with lithium, there was no accumulation of inositol phosphate in response to carbachol in the presence of beryllium at concentrations 100-fold greater than its K_i value (for purified enzyme) in either preparation. BeCl_2 , $\text{Be}_3(\text{PO}_4)_2$, BeCO_3 , BeSO_4 , and BeC_2O_4 were all ineffective at enhancing carbachol-stimulated inositol phosphate accumulation in rat brain slices at concentrations up to 500 μM .

Figure 3 The effect of BeCl_2 and LiCl on carbachol-stimulated $[^3\text{H}]$ inositol phosphate accumulation

Incubation conditions were as described in the Experimental section. (a) Permeabilized SK-N-SH cells where $[\text{carbachol}] = 10$ mM, $[\text{beryllium}] = 100$ μM , and $[\text{lithium}] = 10$ mM. (b) Rat brain cerebral cortical slices where $[\text{carbachol}] = 0.5$ mM, $[\text{beryllium}] = 500$ μM , and $[\text{lithium}] = 10$ mM. The height of each bar represents the mean $\pm$ S.E.M. of three data points. A + signifies presence of compound and – signifies absence of compound in the assay. * indicates significant difference from control; $P < 0.01$, Students *t*-test.

Figure 4 The effect of BeCO_3 , LiCl , $\text{GTP-}\gamma\text{-S}$ and carbachol on $[^3\text{H}]$ inositol phosphate accumulation in non-permeabilized (a) and digitonin-permeabilized (b) SK-N-SH cells

Incubation conditions were as described in the Experimental section. $[\text{GTP-}\gamma\text{-S}] = 100$ μM , $[\text{carbachol}] = 500$ μM , $[\text{GDP-}\beta\text{-S}] = 2$ mM, $[\text{BeCO}_3] = 10$ μM , and $[\text{LiCl}] = 10$ mM. The height of each bar represents the mean $\pm$ S.E.M. of three data points. A + signifies presence of compound and – signifies absence of compound in assay. * indicates significant difference from control; $P < 0.01$ Students *t*-test and $\neq$ indicates significant difference versus $\text{GTP-}\gamma\text{-S}$ + lithium group; $P < 0.02$ *t*-test.

Under conditions where SK-N-SH cells were permeabilized with digitonin, it was thought that a small residue of non-permeabilized cells might predominate over permeabilized cells in producing inositol phosphates. To address this possibility, digitonin-treated and intact cells were exposed to $\text{GTP-}\gamma\text{-S}$ (100 μM) in the presence and absence of LiCl and BeCO_3 (Figures 4a and 4b). In digitonin-permeabilized SK-N-SH cells carbachol, $\text{GTP-}\gamma\text{-S}$, BeCO_3 and LiCl had no significant effect on basal $[^3\text{H}]$ inositol phosphate accumulation. In the presence of LiCl , however, $\text{GTP-}\gamma\text{-S}$ produced a modest but significant increase in $[^3\text{H}]$ inositol phosphate accumulation that was comparable with that of carbachol in the presence of LiCl . The response observed with the combination of $\text{GTP-}\gamma\text{-S}$ and LiCl was completely blocked by co-exposure to $\text{GDP-}\beta\text{-S}$ (2 mM). In contrast, no significant response was observed with $\text{GTP-}\gamma\text{-S}$ in the presence of BeCO_3 at concentrations of 1 μM (results not shown) and 10 μM . In intact SK-N-SH cells, under the same conditions where carbachol produced a robust stimulation of

[³H]inositol phosphate accumulation in the presence of LiCl, GTP- γ -S had no effect on [³H]inositol phosphate accumulation with or without LiCl. Thus, based on these results the differential effects of lithium and beryllium on agonist- or GTP- γ -S-induced phosphatidylinositol turnover are not likely to be due to effects generated in a small fraction of non-permeabilized cells predominating over permeabilized cells.

It was thought that at the concentrations tested beryllium might be toxic to cells and brain slices, and as such would mask any increase in agonist-induced inositol phosphate accumulation. To test this hypothesis, inositol phosphate accumulation in the presence of lithium was studied in the presence of beryllium. As shown in Figure 3, the response to the muscarinic agonist carbachol in the presence of 10 mM lithium was similar whether beryllium was present or not. Moreover, confluent cultures of SK-N-SH cells incubated with 10 μ M BeCl₂ for 2 h excluded Trypan Blue dye to the same extent as cultures not treated with beryllium (> 80 % viability), suggesting that under these conditions beryllium does not cause cell death (results not shown).

DISCUSSION

The results of this study indicate that like the monovalent lithium ion, the bivalent beryllium ion is an inhibitor of human, rat and bovine brain *myo*-inositol monophosphatase. Lithium is an uncompetitive inhibitor of the enzyme [1] and has been shown to bind exclusively to the covalent phosphoenzyme intermediate [25,26]. This is in contrast with beryllium, which as a competitive inhibitor binds only to the free enzyme.

Since beryllium is 5300-fold more potent than lithium as an inhibitor of human *myo*-inositol 1-phosphatase, a comparison of the inhibitory effects of both compounds on endogenous inositol monophosphatase activity in intact preparations was initiated. In permeabilized SK-N-SH cells pre-loaded with either radio-labelled inositol 1-phosphate or inositol 4-phosphate, a time-dependent hydrolysis of these substrates was observed. In this preparation, lithium and beryllium inhibited the hydrolysis of these compounds, indicating that like lithium, beryllium inhibits endogenous *myo*-inositol 1-phosphatase activity. These results indicate that in permeabilized SK-N-SH cells, beryllium appears to gain access to the intracellular pool of *myo*-inositol monophosphatase responsible for the cleavage of both intracellularly generated and exogenously added inositol monophosphates.

The effect of beryllium on agonist-stimulated phosphatidylinositol turnover was also compared with that of lithium in intact SK-N-SH cells and in rat brain cerebral cortical slices pre-labelled with [³H]*myo*-inositol. In these preparations, muscarinic-receptor-stimulated accumulation of inositol monophosphates has been observed only in the presence of lithium [21,23,24]. Beryllium was unable to mimic the effect of lithium in both non-permeabilized SK-N-SH cells (Figure 4a) and in rat brain cerebral cortical slices (Figure 3b). The inability of beryllium to mimic the action of lithium in this assay was unexpected given its potency as an inhibitor of the purified enzyme, and as an inhibitor of inositol monophosphatase exogenously added to permeabilized cells. To assess the possibility that beryllium was inactive in these preparations because of limited cell penetration, a similar experiment was carried out in permeabilized SK-N-SH cells since it was already observed that beryllium potently inhibits endogenous monophosphatase activity in this preparation. However, in permeabilized cells pre-labelled with *myo*-[³H]inositol, muscarinic receptor stimulation of inositol phosphate accumulation was observed only in the presence of lithium, and not in the presence of beryllium (Figure 3a).

Another explanation for the lack of activity of beryllium in

these preparations might be that at the concentrations tested beryllium is cytotoxic. Several experiments were performed to address this possibility. First, permeabilized and non-permeabilized SK-N-SH cells were incubated in the presence and absence of 10 μ M beryllium for 30 min under the same experimental conditions as used for the phosphatidylinositol experiments and then assessed for viability by exclusion of Trypan Blue dye. Results from these experiments revealed that both beryllium-treated and untreated cells were of similar viability (> 80 %). Secondly, agonist-stimulated inositol phosphate accumulation in the presence of lithium was examined in the presence and absence of beryllium in permeabilized SK-N-SH cells (10 μ M) and in rat brain cerebral cortical slices (500 μ M). Under these conditions, beryllium did not affect the response observed in the presence of lithium in either of these preparations. While these results suggest that the inactivity of beryllium on endogenous monophosphatase is not due to problems of toxicity, the possibility still exists that the inactivity of beryllium in intact preparations may be due to permeability problems.

Since the magnitude of receptor-coupled phosphoinositide hydrolysis does not survive permeabilization very well in SK-N-SH cells [cf. Figure 4(a) versus 4(b)], the possibility exists that the reduced signal observed in this preparation is due to a small population of non-permeabilized cells. Given the robust receptor-coupled stimulation of inositol phosphate accumulation in these cells in the presence of lithium, as little as 10 % non-permeabilized cells may be sufficient to produce the observed effects of carbachol. If this were true, then the results with beryllium might still be explained by the possibility that it cannot enter the intact cells predominating in the observed receptor-coupled response. To address this issue, an experiment was designed to examine phosphatidylinositol turnover in permeabilized cells. GTP- γ -S was chosen as an alternate stimulus to carbachol because it was expected that the former would selectively gain access to and stimulate inositol phosphate formation only in permeabilized cells. Indeed GTP- γ -S could only stimulate [³H]inositol phosphate accumulation in the presence of lithium in permeabilized and not in non-permeabilized SK-N-SH cells, and this response was completely blocked by GDP- β -S (Figure 4b). These results indicate that the receptor-coupled response observed in permeabilized cells is due to an interaction with permeabilized cells and does not reflect an interaction with a population of intact cells that survived permeabilization. Thus, in permeabilized cells where access to beryllium is not a problem and under conditions where non-permeabilized cells do not contribute to the pool of [³H]inositol phosphates measured, the differential effects of lithium and beryllium on receptor-coupled inositol phosphate accumulation persists.

One explanation for the lack of effect of beryllium on agonist-induced inositol phosphate accumulation may be that another enzyme that has inositol monophosphatase activity is differentially inhibited by lithium and beryllium. The presence of an enzyme that is not inhibited by beryllium (but is inhibited by lithium) and has a higher K_m value for the substrate inositol 1-phosphate may explain our findings. Thus, when small amounts of exogenous [³H]inositol phosphates were added to permeabilized cell preparations, the enzyme responsible for inositol phosphate hydrolysis was the beryllium-lithium-sensitive enzyme, since the generated inositol phosphate levels would be expected to be low. However, under conditions where inositol phosphate levels rise dramatically (as in the case of agonist-stimulated phosphatidylinositol turnover), the lithium-sensitive, beryllium-insensitive enzyme may then be able to hydrolyse inositol phosphate. If beryllium does not inhibit this enzyme, then inositol phosphate levels may never reach detectable levels.

Figure 5 The effect of competitive and uncompetitive inhibition on the concentration of intermediate B in the reaction pathway $A \rightarrow B \rightarrow C$

This was simulated using equations derived by Cornish-Bowden [27] where $[I]$ is inhibitor concentration, K_i is the inhibition constant, B_0 is the basal concentration of intermediate B and B is the concentration of intermediate B at the respective inhibitor concentration.

Another explanation may be the difference in the mode of enzyme inhibition between beryllium (competitive) and lithium (uncompetitive). Lithium is an uncompetitive inhibitor of inositol monophosphatase and binds only to the enzyme-substrate complex and not to the free enzyme. This is in contrast with a competitive inhibitor which competes with the physiological substrate for the enzyme's active site. In this latter case, as the substrate concentration rises, the effect of the inhibitor is diminished. As discussed by Gee et al. [7] and Cornish-Bowden [27], the nature of inhibition can have enormous consequences on metabolic intermediates in a reaction pathway. As shown in Figure 5, the effect of an uncompetitive inhibitor on the concentration of intermediate B in an $A \rightarrow B \rightarrow C$ reaction is much greater than that of a competitive inhibitor. For example, consider that A is inositol biphosphate, intermediate B is inositol monophosphate and the product C is inositol. At lithium concentrations of 4.5 mM ($[I]/K_i = 10$) and higher, the increase of intermediate B (inositol monophosphate) over basal levels is much greater than 100-fold, whereas at a beryllium concentration of 1 μ M ($[I]/K_i = 10$), the percentage increase of inositol phosphate level over basal is 10-fold (see Figure 5). Thus, the inositol monophosphate concentration will be much greater ($\gg 10$ -fold) in the presence of lithium than beryllium. Sherman et al. [28] have shown that in rats treated with lithium (10 mEq/kg) a 36-fold increase in inositol 1-phosphate level occurs in rat cerebral cortex. The concentration of lithium in rat cortex was found to be 40 mmol/kg dry wt. (~ 40 mM), thus $[I]/K_i = 70$. This is a much smaller increase in inositol 1-phosphate than is predicted by theory (Figure 5). One reason for this small increase may be due to a lithium-insensitive phosphatase that hydrolyses inositol 1-phosphate at a slower rate [7]. Thus, even a competitive inhibitor (e.g. beryllium) at 100-fold its K_i value would never attain levels of inositol 1-phosphate necessary to detect accumulation. The importance of this observation is not trivial since all competitive inhibitors of inositol phosphatase may have to reach $[I]/K_i$ levels of 1×10^4 – 1×10^6 in order for any lithiummimetic

activity to be observed. More potent competitive inhibitors of *myo*-inositol monophosphatase will have to be discovered in order to test this hypothesis. Therefore, potent uncompetitive inhibitors may be the goal in the discovery of inositol monophosphatase inhibitors as replacements for lithium.

In summary, it appears that inhibition of inositol monophosphatase is not sufficient to show a lithiummimetic effect in SK-N-SH cells and in rat brain cortical slices. Beryllium salts are potent, competitive inhibitors of partially purified *myo*-inositol monophosphatase and completely prevent hydrolysis of exogenously supplied [^{3} H]inositol phosphates at concentrations of 1–10 μ M. However, in contrast with lithium, agonist-induced accumulation of inositol phosphates does not occur in the presence of beryllium. The reason for this anomaly may be the nature of inhibition (competitive for beryllium; uncompetitive for lithium) or a different monophosphatase that is inhibited by lithium but not by beryllium.

We are grateful to Dr. R. M. Snider, Dr. R. W. Spencer, Dr. Frank Menniti, Dr A. Nagel, W. A. Fairman and J. L. Collins for helpful discussions and/or technical assistance.

REFERENCES

- Hallcher, L. M. and Sherman, W. R. (1980) *J. Biol. Chem.* **255**, 10896–10901
- Sherman, W. R., Gish, B. G., Honchar, M. P. and Munsell, L. Y. (1986) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **45**, 2639–2646
- Majerus, P. W., Connolly, T. M., Bansal, V. S., Inhorn, R. C., Ross, T. S. and Lips, D. L. (1988) *J. Biol. Chem.* **263**, 3051–3054
- Berridge, M. J. (1987) *Annu. Rev. Biochem.* **58**, 159–193
- Chen, I. W. and Charalampous, F. C. (1966) *J. Biol. Chem.* **241**, 2194–2199
- Attwood, P. V., Ducep, J. B. and Chanal, M. C. (1988) *Biochem. J.* **253**, 387–394
- Gee, N. S., Ragan, C. I., Watling, K. J., Aspley, S., Jackson, R. G., Reid, G. G., Gani, D. and Shute, J. K. (1988) *Biochem. J.* **249**, 883–889
- Takimoto, K., Okada, M., Matsuda, Y. and Nakagawa, H. (1985) *J. Biochem.* **98**, 363–370
- Ganzhorn, A. J. and Chanal, M. C. (1990) *Biochemistry* **29**, 6065–6071
- Allison, J. H. and Stewart, M. A. (1971) *Nature (London)* **233**, 267–268
- Allison, J. H., Blisner, M. E., Holland, W. H., Hips, P. P. and Sherman, W. R. (1976) *Biochem. Biophys. Res. Commun.* **71**, 664–670
- Berridge, M. J. (1989) *J. Am. Med. Assoc.* **262**, 1834–1841
- Baraban, J. M., Worley, P. F. and Snyder, S. H. (1989) *Am. J. Psychiatry* **146**, 1251–1259
- Bunney, W. E. and Garland-Bunney, B. L. in *Psychopharmacology: The Third Generation of Progress* (Meltzer, H. Y., ed.), pp. 553–565. Raven Press, New York
- Avissar, S., Schreiber, G., Danon, A. and Belmaker, R. H. (1988) *Nature (London)* **331**, 440–442
- Godfrey, P. P., McClue, S. J., White, A. M., Wood, A. J. and Grahame-Smith, D. G. (1989) *J. Neurochem.* **52**, 498–506
- Klemperer, F. W., Miller, J. M. and Hill, C. J. (1949) *J. Biol. Chem.* **180**, 281–288
- Klemperer, F. W. (1950) *Science* **112**, 423
- Lanzetta, P. A., Alvarez, L. J., Reinach, P. S. and Candia, O. A. (1979) *Anal. Biochem.* **100**, 95–97
- Cleland, W. W. (1979) *Methods Enzymol.* **63**, 103–138
- Fisher, S. K. and Snider, R. M. (1987) *Mol. Pharmacol.* **32**, 81–90
- Fisher, S. K., Domask, L. M. and Roland, R. M. (1989) *Mol. Pharmacol.* **35**, 195–204
- Zorn, S. H., Duman, R. S., Krogsgaard-Larsen, P., Micheletti, R., Giraldo, E., Giachetti, A. and Enna, S. J. (1987) *J. Pharmacol. Exp. Ther.* **242**, 173–178
- Brown, E. B., Kendall, D. A. and Nahorski, S. R. (1984) *J. Neurochem.* **42**, 1379–1387
- Jackson, R. G., Gee, N. S. and Ragan, C. I. (1989) *Biochem. J.* **264**, 419–422
- Shute, J. K., Baker, R., Billington, D. C. and Gani, D. (1988) *J. Chem. Soc., Chem. Commun.* 626–628
- Cornish-Bowden, A. (1986) *FEBS Lett.* **203**, 3–6
- Sherman, W. R., Munsell, L. Y., Gish, B. G. and Honchar, M. P. (1985) *J. Neurochem.* **44**, 798–807