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Noncompetitive Inhibition of Inositol Monophosphatase by K-76 Monocarboxylic Acid

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SUMMARY

K-76COONa, a fungal product that was previously isolated for its inhibition of complement activation, was found to inhibit *myo*-inositol monophosphatase activity. K-76COONa was slightly more potent than lithium, with a K_i of approximately 0.5 mM. Kinetic analyses with D-*myo*-inositol 1-phosphate as the substrate showed that *myo*-inositol monophosphatase inhibition by K-76COONa was noncompetitive relative to substrate but competitive with activation by magnesium. Higher concentrations of K-76COONa were necessary to inhibit *myo*-[^{3}H]inositol 1,4-bisphosphate hydrolysis by inositol 1,4-bisphosphate/inositol 1,3,4-trisphosphate 1-phosphatase (IC_{50} = approximately 7.5 mM). K-76COONa may be useful for further investigation of the mechanism of *myo*-inositol monophosphatase and for determination of whether inhibition of this enzyme plays a role in the therapeutic effectiveness of lithium in treatment of affective disorders.

The enzyme *myo*-inositol monophosphatase (EC 3.1.3.25) dephosphorylates both enantiomers of Ins(1)P and Ins(4)P to free *myo*-inositol (1) and appears to be a key enzyme in the recycling of inositol polyphosphate second messengers to regenerate phosphatidylinositol and polyphosphoinositides. This enzyme may be especially important for the generation of inositol in the brain, because transport of dietary inositol across the blood-brain barrier is limited (2, 3).

Inositol monophosphatase has become the subject of great interest since Naccarato *et al.* (4) demonstrated that it is sensitive to inhibition by lithium, and it was suggested that this enzyme may be the site of the therapeutic action of lithium in the treatment of manic-depressive illness (5). Although substantial evidence has accumulated to support the hypothesis that this enzyme is the important target for lithium action (6–9) (but see also Ref. 10), other actions of lithium, including effects on neurotransmitter receptors (11, 12), guanine nucleotide-binding regulatory proteins (13), and cation transport (14), have also been proposed to mediate the effectiveness of lithium in affective disorders.

More definitive determination of whether inositol monophosphatase is the primary site of the therapeutic action of lithium could come from studies with more specific inhibitors of this enzyme. Recently, there was a preliminary report that a fungal compound that is structurally similar to the complement inhibitor K-76COONa (15) inhibits inositol monophosphatase activity,[1] although the mechanism of action was not reported. The present paper shows that K-76COONa inhibits inositol monophosphatase activity by a mechanism that is noncompetitive with substrate and competitive with magnesium activation. Furthermore, a second lithium-sensitive phosphatase, Ins(1,4)P_2ase, which dephosphorylates Ins(1,4)P_2 and inositol 1,3,4-trisphosphate to Ins(4)P and inositol 3,4-bisphosphate, respectively (16, 17), is shown to be inhibited by higher concentrations of K-76COONa.

Experimental Procedures

Materials. Frozen bovine brains (350–450 g) were purchased from Pel-Freez (Rogers, AR). Polyvinyl alcohol (98% hydrolyzed; M_r 11,000–31,000) was from Aldrich Chemical Co. (Milwaukee, WI). K-76COONa was obtained from Otsuka Pharmaceutical Co., Ltd. (Tokushima, Japan). The K-76COONa was analyzed for contaminating lithium content by atomic absorption (Galbraith Laboratories, Knoxville, TN), and duplicate determinations showed that the compound contained <0.08% lithium by weight. D-[2-^{3}H]Ins(1,4)P_2 was purchased from Amersham Corp. (Arlington Heights, IL). AG 1-X8 resin was from Bio-Rad Laboratories (Richmond, CA). Malachite green, D-Ins(1)P (95%

[1] M. Meinz, L. Huang, L. Kong, L. Guariglia, R.A. Giacobbe, T. Lam, S. Mochales, J. Liesch, R.W. Burg, R.L. Monaghan, R. Ransom, and I. Ragan. Discovery of L-920,142, an inhibitor of Inositol-1-Phosphatase from the novel *Memnoniella sp.* Presented at the 90th Annual Meeting of the American Society for Microbiology, Anaheim CA, May 13–17, 1990.

ABBREVIATIONS: Ins(1)P, *myo*-inositol 1-phosphate; K-76COONa, sodium 6,7-dihydroxy-2,5,5,8a-tetramethyl-1,2,3,4,4a,5,6,7,8,8a-decahydronaphthalene-1-spiro-2′-(7′-carboxylate-6′-formyl-4′-hydroxy-2′,3′-dihydrobenzofuran); Ins(4)P, *myo*-inositol 4-phosphate; Ins(1,4)P_2, *myo*-inositol 1,4-bisphosphate; Ins(1,4)P_2ase, inositol 1,4-bisphosphate/inositol 1,3,4-trisphosphate 1-phosphatase; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid; InsP_1, inositol monophosphate.

pure), D-Ins(1,4)P_2, and all other chemicals were from Sigma Chemical Co. (St. Louis, MO).

Inositol monophosphatase preparation. Inositol monophosphatase was partially purified by an abbreviation of the procedure of Meek *et al.* (18). A frozen bovine brain was thawed, cut into pieces, homogenized in a blender in 2 volumes of homogenization buffer containing 50 mM Tris·HCl, pH 7.5, 150 mM KCl, and 0.1 mM EGTA, and centrifuged at 15,000 × *g* for 30 min. Ammonium sulfate (242 g/liter) was added to the decanted supernatant, and the mixture was stirred for 30 min at 4° and then recentrifuged at 15,000 × *g* for 30 min. Additional ammonium sulfate (130 g/liter) was added to the supernatant, and the mixture was stirred and centrifuged as before. The resulting pellet was dissolved in homogenization buffer (93 ml/kg of brain weight), and EDTA and dithiothreitol were added to a final concentration of 1 mM each. This mixture was heated in a preheated 75° water bath for 1 hr and centrifuged at 15,000 × *g* for 30 min, and fresh dithiothreitol was again added to 1 mM concentration. The solution was dialyzed overnight against 2 liters of 50 mM Tris·HCl, pH 7.5, at 4°, dialyzed again for at least 6 hr, and stored in aliquots at −70° until use.

Inositol monophosphatase assay. Enzyme assays were done in 96-well microtiter dishes. To each well, 20 μl of D-Ins(1)P, 30 μl of K-76COONa, and 50 μl of 3× buffer containing 150 mM Tris·HCl, pH 7.5, 0.75 M KCl, and 9 mM $MgCl_2$ (except in Mg^{2+} dose-response experiments) were added, to yield appropriate final concentrations in a final volume of 150 μl. Inositol monophosphatase was diluted 1/150 in H_2O and equilibrated with the microtiter dishes at 37° for 15 min. The reaction was begun by addition of 50 μl of diluted enzyme to each well, and dishes were incubated at 37° for 1 hr.

Inorganic phosphate produced by the inositol monophosphatase reaction was measured by the procedure of Van Veldhoven and Mannaerts (19), adapted for the microtiter dish format. The reaction was terminated by addition of 30 μl of 1.75% (w/v) ammonium heptamolybdate·4 H_2O in 6.3 N H_2SO_4. After 10 min at room temperature, 30 μl of 0.035% (w/v) malachite green, 0.35% (w/v) polyvinyl alcohol, were added to each well, and the plate was allowed to sit at room temperature for 30 min before determination of the absorbance at 650 nm on a microplate reader. Phosphate concentrations were calculated relative to a KH_2PO_4 standard curve. Control wells in which the enzyme was added immediately after ammonium molybdate/H_2SO_4 were routinely assayed, and the phosphate in these wells was subtracted from that of the corresponding reaction wells.

Ins(1,4)P_2ase assay. Ins(1,4)P_2ase was prepared from bovine brain, as described above for inositol monophosphatase, except that the 1-hr heat-denaturation treatment was omitted. Therefore, this crude enzyme preparation also had monophosphatase activity.

Enzyme activity was measured by a modification of the procedure of Ragan *et al.* (17). Reaction tubes each contained a final volume of 0.1 ml of the following assay mixture: 250 mM KCl, 50 mM Tris·HCl, pH 7.5, 3 mM $MgCl_2$, 0.1 mM D-Ins(1,4)P_2 containing 25 nCi of D-[^{3}H] Ins(1,4)P_2, and enzyme preparation that was diluted to hydrolyze less than 35% of the Ins(1,4)P_2. The reaction was initiated by addition of the enzyme, allowed to proceed for 15 min at 37°, and terminated by addition of 20 μl of 10% (w/v) trichloroacetic acid. Contents of each tube were diluted with 5 ml of H_2O and applied to AG 1-X8 (formate form) columns. Free inositol was collected as the 5-ml sample elution plus a subsequent elution with 3 ml of H_2O. Columns were then washed with 5 ml of H_2O, followed by elution of inositol monophosphates with 8 ml of 0.2 M ammonium formate/0.1 M formic acid. Radioactivity was determined by scintillation counting. This elution protocol yielded 99.5 and 92.4% recovery of inositol and inositol monophosphates respectively, without contamination by unhydrolyzed inositol bisphosphates.

Results

Inhibition of Ins(1)P hydrolysis. Ins(1)P dephosphorylation by the inositol monophosphatase preparation used in these studies was inhibited by 91% by 10 mM LiCl (data not shown), indicating that the inositol monophosphatase activity is predominantly the previously described lithium-sensitive enzyme (1, 4, 18, 20). K-76COONa inhibited inositol monophosphatase activity in a dose-dependent manner, with an IC_{50} of 0.45 mM (Fig. 1). Phosphatase activity was inhibited by 82% by 3 mM K-76COONa; higher drug concentrations could not be tested due to limited solubility.

Fig. 2 shows the inhibition of inositol monophosphatase activity by various concentrations of K-76COONa in the presence of increasing substrate concentrations. The lower apparent V_{max} values attained with higher concentrations of K-76COONa are suggestive of a mechanism of inhibition other than competition with substrate. The Lineweaver-Burk transformations shown in Fig. 2B indicate a noncompetitive mode of inhibition relative to Ins(1)P concentration. The K_i for K-76COONa was approximately 0.5 mM.

Competition with magnesium. Lineweaver-Burk plots of enzyme inhibition by fixed concentrations of K-76COONa in the presence of increasing Mg^{2+} concentrations showed a common intersection on the *y*-axis, suggesting that inhibition by K-76COONa is competitive with magnesium (Fig. 3). In the presence of higher concentrations of K-76COONa, double-reciprocal plots with varying [Mg^{2+}] showed upward concavity. This may be due to cooperativity of magnesium binding or partial inhibition of the enzyme by higher magnesium concentrations, as has been previously reported (20, 21).

Inhibition of Ins(1,4)P_2 hydrolysis. Because hydrolysis of Ins(1,4)P_2 by Ins(1,4)P_2ase has also been shown to be inhibited by lithium, the effect of K-76COONa on Ins(1,4)P_2 hydrolysis was measured. Ins(1,4)P_2 dephosphorylation, measured as the sum of inositol and $InsP_1$ production, was inhibited by K-76COONa in a dose-dependent manner (Fig. 4). Although maximal inhibition of Ins(1,4)P_2 hydrolysis was not achieved, due to limited drug solubility, K-76COONa appeared to be 1 order of magnitude less potent in inhibition of Ins(1,4)P_2ase activity (IC_{50} = approximately 7 mM) than in inhibition of inositol monophosphatase.

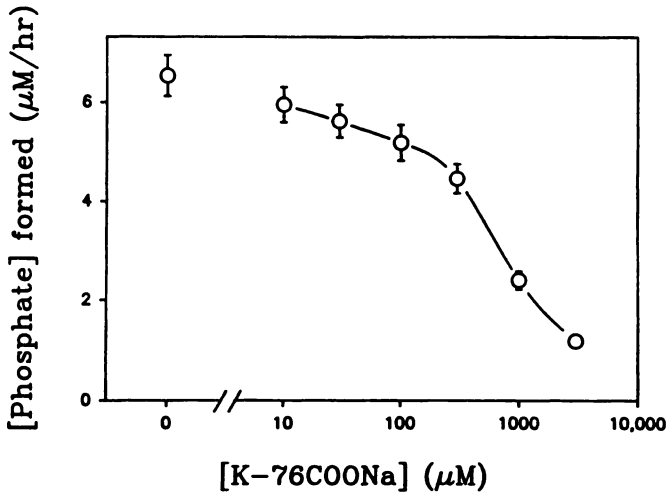

Fig. 1. Inhibition of inositol monophosphatase activity by K-76COONa. Enzyme activity was measured for 1 hr at 37° in the presence of increasing concentrations of K-76COONa, with 0.3 mM D-Ins(1)P as substrate and 3 mM Mg^{2+}. Each value represents the mean ± standard error of four determinations from two experiments performed in duplicate. For *points* without *error bars*, the standard error was within the size of the symbol.

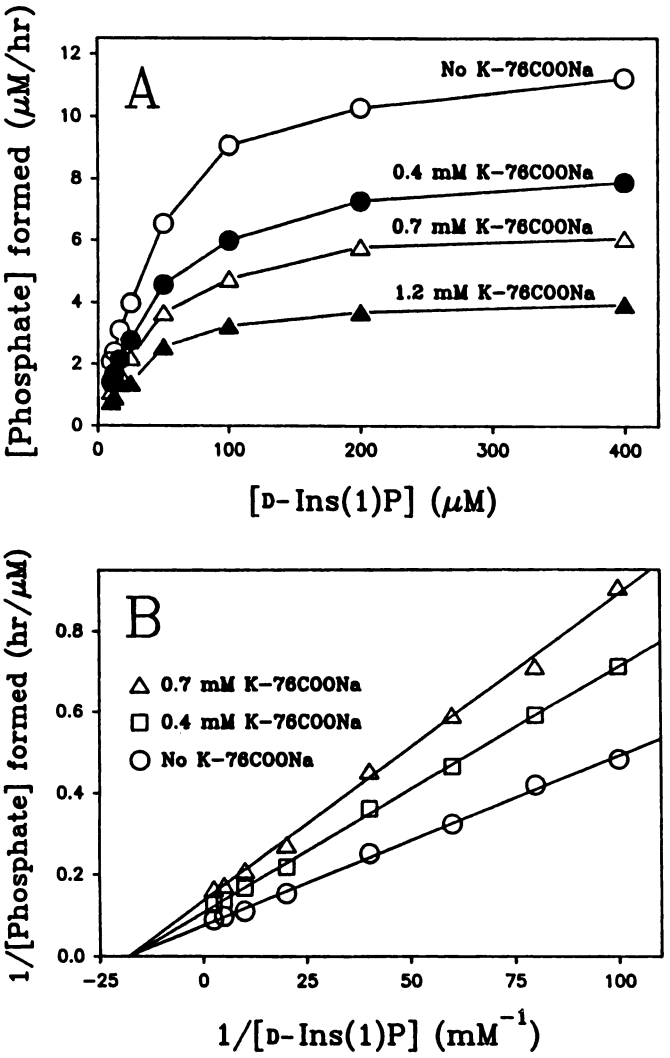

Fig. 2. Kinetics of inhibition of inositol monophosphatase by K-76COONa, as a function of substrate concentration. Measurements were made in the presence of 3 mM Mg^{2+}. A, Michaelis-Menten plots of enzyme activity in the presence of fixed concentrations of K-76COONa. B, Lineweaver-Burk plots with fixed K-76COONa concentrations. *Lines* were fitted by linear regression. The convergence of the lines on the *x*-axis indicates noncompetitive inhibition by K-76COONa relative to D-Ins(1)P. K_i was calculated to be approximately 0.5 mM K-76COONa. Each *point* is the mean of duplicate determinations. Data are representative of three separate experiments.

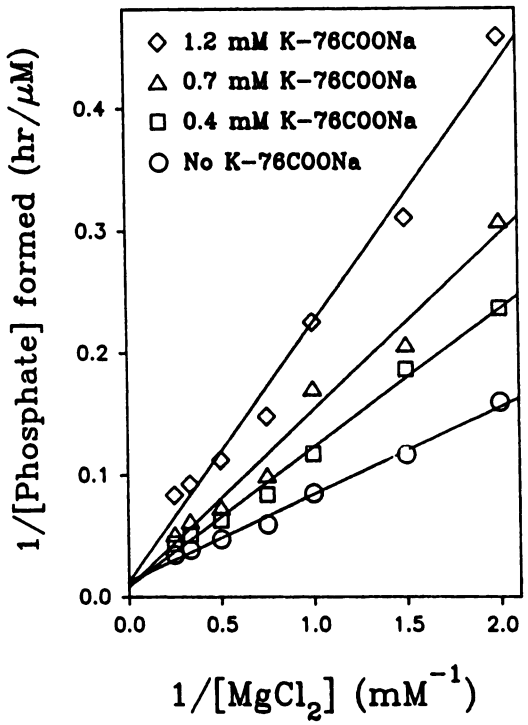

Fig. 3. Lineweaver-Burk plots of inhibition of inositol monophosphatase by K-76COONa as a function of Mg^{2+} concentration. Measurements were made in the presence of 0.3 mM D-Ins(1)P as substrate. *Lines* in the presence of fixed concentrations of K-76COONa were fitted by linear regression. Convergence on the *y*-axis indicates competitive inhibition by K-76COONa relative to Mg^{2+} concentration. Each *point* represents the mean of duplicate determinations. Data are representative of two separate experiments.

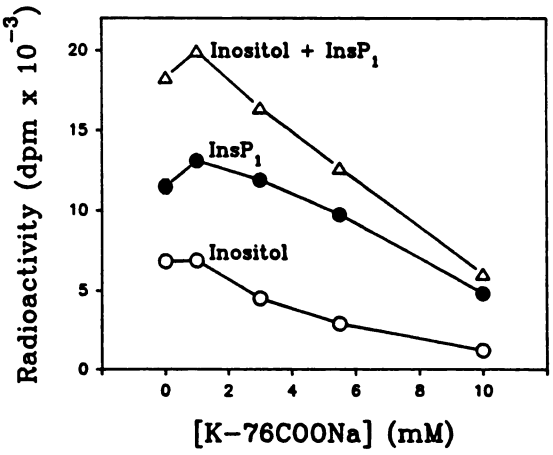

Fig. 4. Inhibition of Ins(1,4)P_2 hydrolysis by K-76COONa. Enzyme activity was measured for 15 min at 37° in the presence of increasing concentrations of K-76COONa, with 0.1 mM D-[^{3}H]Ins(1,4)P_2 (58,966 dpm/tube) as substrate. The sum of [^{3}H]inositol and [^{3}H]InsP_1s formed indicates the amount of [^{3}H]Ins(1,4)P_2 hydrolyzed. Each *point* is the mean of duplicate determinations. Data are representative of results obtained in two separate experiments.

Discussion

K-76COONa was found to inhibit inositol monophosphatase noncompetitively relative to substrate. Because K-76COONa lacks a cationic moiety, its competition with Mg^{2+} may result from masking of the Mg^{2+} binding site, rather than direct interaction with an anionic binding site. K-76COONa was also found to inhibit the hydrolysis of Ins(1,4)P_2 by Ins(1,4)P_2ase, another Li^+-sensitive, Mg^{2+}-dependent phosphatase in the inositol polyphosphate metabolic pathway.

The possibility that K-76COONa inhibits inositol monophosphatase and Ins(1,4)P_2ase activities by simple chelation of Mg^{2+} can be ruled out by consideration of the differential potencies of K-76COONa in inhibiting the two enzymes. Although similar concentrations of Mg^{2+} are required for half-maximal activation of both enzymes [K_m for inositol monophosphatase = 0.2–1.0 mM (20, 21); K_m for Ins(1,4)P_2ase = 0.3 mM (16)], at least 15 times more K-76COONa is required for half-maximal inhibition of Ins(1,4)P_2ase (IC_{50} = 7.5 mM) than for half-maximal inhibition of inositol monophosphatase (IC_{50} = 0.45 mM) under identical buffering conditions (pH 7.5 with 3 mM Mg^{2+}). Thus, the greater potency of K-76COONa in inhibiting inositol monophosphatase indicates that K-76COONa must interact directly with this enzyme, rather

than simply removing the Mg^{2+} cofactor. Additionally, the carboxylic acid moiety of K-76COONa, which is likely to be the only anionic moiety at pH 7.5 that could chelate a cation, does not appear to be necessary for inhibition of inositol monophosphatase activity, because the closely related compound L-920,142 lacks this carboxylic acid group but shows similar potency in inhibition of inositol monophosphatase.[1]

K-76COONa has also been shown previously to inhibit complement-mediated hemolysis, predominantly through combination with C5 and prevention of its activation to C5b (15, 22). Interestingly, the anticomplementary action of K-76COONa also appears to occur through inhibition of a Mg^{2+}-dependent reaction (22). Furthermore, the IC_{50} (340 μM) for inhibition of complement-mediated lysis (15) is similar to that reported here for inhibition of inositol monophosphatase (450 μM). Thus, similar mechanisms may be involved in the inhibition of both of these activities by K-76COONa.

To assure that the inhibitory activity of K-76COONa is not the result of contaminating lithium in the drug preparation, elemental analysis of K-76COONa was performed (see Experimental Procedures). Atomic absorption showed that the K-76COONa contains less than 0.08% lithium. At the K_i of K-76COONa for inositol monophosphatase inhibition (0.5 mM), this would correspond to a maximum lithium concentration of less than 25 μM, which is 40-fold lower than the reported K_i of 1 mM for lithium inhibition of the monophosphatase (1, 21, 23). This, coupled with the observation that K-76COONa inhibits inositol monophosphatase noncompetitively, whereas lithium inhibits by an uncompetitive mechanism, demonstrates that the inhibitory activity of K-76COONa is not the result of lithium contamination.

The effectiveness of lithium in inhibiting inositol monophosphatase activity is thought to be related to its uncompetitive mechanism of action (1), because in an intact cell a competitive inhibitor would probably be rapidly overcome by build-up of substrate levels. Similarly, the noncompetitive mechanism of inhibition by K-76COONa should make it a more resilient inhibitor and, thus, a more useful tool for determination of whether inhibition of inositol monophosphatase is an effective strategy for treatment of affective disorders such as manic-depressive illness.

To date, reported inhibitors of inositol monophosphatase fall into two categories, inorganic ions and competitive monophosphates. In addition to lithium, numerous other ions inhibit this enzyme. Because the enzyme has a requirement for magnesium, divalent cations such as Ca^{2+}, Cu^{2+}, Mn^{2+}, and Zn^{2+} inhibit activity by displacing Mg^{2+} (1, 20, 23). Anions such as sulfate and fluoride also inhibit inositol monophosphatase activity (20), perhaps by binding to magnesium in the active site of the enzyme (21). Unfortunately, the effects of these simple ions are not selective for this enzyme, because inorganic ions play a ubiquitous role in the regulation of a large variety of enzymes and transporters. Phosphate and monophosphates such as glycerol 2-phosphate, 2′-AMP, inositol 2-phosphate, and deoxy derivatives of Ins(1)P inhibit dephosphorylation of Ins(1)P and Ins(4)P by competition for the substrate binding site (1, 20, 24, 25) and are, therefore, of limited utility, as discussed above.

Based on the observations that the enzymes Ins(1,4)P_2ase and inositol monophosphatase play sequential roles in the metabolism of Ins(1,4)P_2 to inositol, are both Mg^{2+} dependent, and are both uncompetitively inhibited by lithium, it has been suggested that these two enzymes are related members of an enzyme family (16). The finding that both enzymes are inhibited by K-76COONa may further support this hypothesis. Recently, cDNA clones for both inositol monophosphatase and Ins(1,4)P_2ase have been isolated and sequenced (26, 27). The deduced amino acid sequences showed only minimal homology (27). A recent report demonstrating that enzymes in a common catabolic pathway with only minor homology in primary amino acid sequence can have virtually identical three-dimensional structure (28) might suggest an interesting hypothesis to explain the similarities in the regulation of inositol monophosphatase and Ins(1,4)P_2ase.

In conclusion, K-76COONa is the first organic inhibitor of *myo*-inositol monophosphatase reported to act through a mechanism other than competition with substrate. As such, K-76COONa may be useful in delineating whether inhibition of this enzyme is involved in the therapeutic effectiveness of lithium.

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References

1. Gee, N. S., C. I. Ragan, K. J. Watling, S. Aspley, R. G. Jackson, G. G. Reid, D. Gani, and J. K. Shute. The purification and properties of *myo*-inositol monophosphatase from bovine brain. *Biochem. J.* **249**:883–889 (1988).
2. Margolis, R. U., R. Press, N. Altszuler, and M. A. Stewart. Inositol production by the brain in normal and alloxan-diabetic dogs. *Brain Res.* **28**:535–539 (1971).
3. Spector, R., and A. V. Lorenzo. *Myo*-inositol transport in the central nervous system. *Am. J. Physiol.* **228**:1510–1518 (1975).
4. Naccarato, W. F., R. E. Ray, and W. W. Wells. Biosynthesis of *myo*-inositol in rat mammary gland: isolation and properties of the enzymes. *Arch. Biochem. Biophys.* **164**:194–201 (1974).
5. Berridge, M. J., C. P. Downes, and M. R. Hanley. Lithium amplifies agonist-dependent phosphatidylinositol responses in brain and salivary glands. *Biochem. J.* **206**:587–595 (1982).
6. Sherman, W. R., L. Y. Munsell, B. G. Gish, and M. P. Honchar. Effects of systemically administered lithium on phosphoinositide metabolism in rat brain, kidney, and testis. *J. Neurochem.* **44**:798–807 (1985).
7. Worley, P. F., W. A. Heller, S. H. Snyder, and J. M. Barbaran. Lithium blocks a phosphoinositide-mediated cholinergic response in hippocampal slices. *Science (Washington D. C.)* **239**:1428–1429 (1988).
8. Kennedy, E. D., R. A. J. Challiss, C. I. Ragan, and S. R. Nahorski. Reduced inositol polyphosphate accumulation and inositol supply induced by lithium in stimulated cerebral cortex slices. *Biochem. J.* **267**:781–786 (1990).
9. Joseph, N. E., P. F. Renshaw, and J. S. Leigh. Systemic lithium administration alters rat cerebral cortex phospholipids. *Biol. Psychiatry* **22**:540–544 (1987).
10. Honchar, M. P., K. E. Ackermann, and W. R. Sherman. Chronically administered lithium alters neither *myo*-inositol monophosphatase activity nor phosphoinositide levels in rat brain. *J. Neurochem.* **53**:590–594 (1989).
11. Rosenblatt, J. E., C. B. Pert, J. F. Tallman, A. Pert, and W. E. Bunney. The effect of imipramine and lithium on α- and β-receptor binding in rat brain. *Brain Res.* **160**:186–191 (1979).
12. Maggi, A., and S. J. Enna. Regional alterations in rat brain neurotransmitter systems following chronic lithium treatment. *J. Neurochem.* **34**:888–892 (1980).
13. Avissar, S., G. Schreiber, A. Danon, and R. H. Belmaker. Lithium inhibits adrenergic and cholinergic increases in GTP binding in rat cortex. *Nature (Lond.)* **331**:440–442 (1988).
14. Wood, A. J., M. Elphick, and D. G. Grahame-Smith. Effect of lithium and of other drugs used in the treatment of manic illness on the cation-transporting properties of Na^+,K^+-ATPase in mouse brain synaptosomes. *J. Neurochem.* **52**:1042–1049 (1989).
15. Miyazaki, W., H. Tamaoka, M. Shinohara, H. Kaise, T. Izawa, Y. Nakano, T. Kinashita, K. Hong, and K. Inoue. A complement inhibitor produced by *Stachybotrys complementi, nov sp. K-76*, a new species of fungi imperfecti. *Microbiol. Immunol.* **24**:1091–1108 (1980).
16. Inhorn, R. C., and P. W. Majerus. Inositol polyphosphate 1-phosphatase from calf brain: purification and inhibition by Li^+, Ca^{2+}, and Mn^{2+}. *J. Biol. Chem.* **262**:15946–15952 (1987).
17. Ragan, C. I., K. J. Watling, N. S. Gee, S. Aspley, R. G. Jackson, G. G. Reid, R. Baker, D. C. Billington, R. J. Barnaby, and P. D. Leeson. The dephosphorylation of inositol 1,4-bisphosphate to inositol in liver and brain involves two distinct Li^+-sensitive enzymes and proceeds via inositol 4-phosphate. *Biochem. J.* **249**:143–148 (1988).

18. Meek, J. L., T. J. Rice, and E. Anton. Rapid purification of inositol monophosphate phosphatase from beef brain. *Biochem. Biophys. Res. Commun.* **156**:143–148 (1988).
19. Van Veldhoven, P. P., and G. P. Mannaerts. Inorganic and organic phosphate measurements in the nanomolar range. *Anal. Biochem.* **161**:45–48 (1987).
20. Hallcher, L. M., and W. R. Sherman. The effects of lithium ion and other agents on the activity of *myo*-inositol-1-phosphatase from bovine brain. *J. Biol. Chem.* **255**:10896–10901 (1980).
21. Ganzhorn, A. J., and M.-C. Chanal. Kinetic studies with *myo*-inositol monophosphatase from bovine brain. *Biochemistry* **29**:6065–6071 (1990).
22. Redelman, D., and D. Hudig. The mechanism of cell-mediated cytotoxicity. IV. K-76 COONa, which inhibits the activity of factor I and of C5, inhibits early events in cytotoxic T-lymphocyte-mediated cytolysis and in T-lymphocyte activation. *Cell. Immunol.* **88**:16–28 (1984).
23. Takimoto, K., M. Okada, Y. Matsuda, and H. Nakagawa. Purification and properties of *myo*-inositol-1-phosphatase from rat brain. *J. Biochem.* **98**:363–370 (1985).
24. Baker, R., J. J. Kulagowski, D. C. Billington, P. D. Leeson, I. C. Lennon, and N. Liverton. Synthesis of 2- and 6-deoxyinositol 1-phosphate and the role of the adjacent hydroxy groups in the mechanism of inositol monophosphatase. *J. Chem. Soc. Chem. Commun.* 1383–1385 (1989).
25. Baker, R., P. D. Leeson, N. J. Liverton, and J. J. Kulagowski. Identification of (1*S*)-phosphoryloxy-(2*R*,4*S*)-dihydroxycyclohexane as a potent inhibitor of inositol monophosphatase. *J. Chem. Soc. Chem. Commun.* 462–464 (1990).
26. Diehl, R. E., P. Whiting, J. Potter, N. Gee, C. I. Ragan, D. Linemeyer, R. Schoepfer, C. Bennett, and R. A. F. Dixon. Cloning and expression of bovine brain inositol monophosphatase. *J. Biol. Chem.* **265**:5946–5949 (1990).
27. York, J. D., and P. W. Majerus. Isolation and heterologous expression of a cDNA encoding bovine inositol polyphosphate 1-phosphatase. *Proc. Natl. Acad. Sci. USA* **87**:9548–9552 (1990).
28. Neidhart, D. J., G. L. Kenyon, J. A. Gerlt, and G. A. Petsko. Mandelate racemase and muconate lactonizing enzyme are mechanistically distinct and structurally homologous. *Nature (Lond.)* **347**:692–694 (1990).

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