Formation of Inositol 1,4,5-Trisphosphate and Inositol 1,3,4-Trisphosphate from Inositol 1,3,4,5-Tetrakisphosphate and Their Pathways of Degradation in RBL-2H3 Cells*

Jose R. Cunha-Melo, Nicholas M. Dean, Hydar Ali, and Michael A. Beaven

From the Laboratory of Chemical Pharmacology, National Heart, Lung, and Blood Institute and the §Laboratory of Biological Chemistry, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892

Previous studies with antigen-stimulated rat basophilic leukemia (RBL-2H3) cells indicated the formation of multiple isomers of each of the various categories of inositol phosphates. The identities of the different isomers have been elucidated by selective labeling of [3H]inositol 1,3,4,5-tetrakisphosphate with [32P]phosphate in the 3'– or 4',5'-positions and by following the metabolism of different radiolabeled inositol phosphates in extracts of RBL-2H3 cells. We report here that inositol 1,3,4,5-tetrakisphosphate, when incubated with the membrane fraction of extracts of RBL-2H3 cells, was converted to inositol 1,4,5-trisphosphate and inositol 1,3,4-trisphosphate. Further dephosphorylation of the inositol polyphosphates proceeded rapidly in whole extracts of cells, although the process was significantly retarded when ATP (2 mM) levels were maintained by an ATP-regenerating system. The degradation of inositol 1,4,5-trisphosphate proceeded with the sequential formation of inositol 1,4-bisphosphate, the inositol 4-monophosphate (with smaller amounts of the 1-monophosphate), and finally inositol. Inositol 1,3,4-trisphosphate, on the other hand, was converted to inositol 1,3,bisphosphate and inositol 3,4-bisphosphate and subsequently to inositol 4-monophosphate and inositol 1-monophosphate (stereoisomeric forms were undetermined). The possible implications of the apparent interconversion between inositol 1,4,5-trisphosphate and inositol 1,3,4,5-tetrakisphosphate in regulating histamine secretion in the RBL-2H3 cells are discussed.

Previous studies with [3H]inositol-labeled rat basophilic leukemia (RBL-2H3) cells, which secrete histamine when stimulated with antigen (1), showed that antigen stimulation resulted in the production of three isomers of inositol monophosphate and three isomers of inositol bisphosphate in addition to inositol 1,4,5-trisphosphate, inositol 1,3,4-trisphosphate and inositol 1,3,4,5-tetrakisphosphate (2). The inositol monophosphates were identified as the 1-phosphate, 2-phosphate, and 4-phosphate esters of inositol by their coelution with the authentic labeled compounds on HPLC columns. Inositol 1,4-bisphosphate was identified in a similar manner; the other two bisphosphate isomers were not identified although they did not co-elute with the two authentic inositol bisphosphate standards (2,4- and 4,5-bisphosphate) that were available to us at that time.

The studies with intact cells did not clearly define the precise order in which the different inositol phosphates were generated after antigen stimulation but additional studies with permeabilized RBL-2H3 cells indicated that, as in other cells (3–7), inositol 1,4,5-trisphosphate was converted by a soluble 3'-kinase to inositol 1,3,4,5-tetrakisphosphate and then to inositol 1,3,4-trisphosphate by the action of a membrane inositol 5'-phosphomonoesterase (2).

The multiple isomers of inositol bisphosphate and inositol monophosphate in intact cells could indicate degradation of the more highly phosphorylated inositol metabolites through more than one pathway. In attempting to elucidate these pathways we found unexpectedly that [3H]inositol 1,3,4,5-tetrakisphosphate, when incubated with the membrane fraction of extracts of RBL-2H3 cells in the presence of ATP, was converted to both [3H]inositol 1,4,5-trisphosphate and [3H]inositol 1,3,4-trisphosphate. We describe here the identification of these two isomers, as well as that of the previously unidentified isomers of [3H]inositol bisphosphate, by selectively labeling the 3-position of [3H]inositol 1,3,4,5-tetrakisphosphate with [32P]phosphate for some experiments and the 4- and 5-positions for other experiments.

MATERIALS AND METHODS

General Procedures—The RBL-2H3 cells were grown in suspension culture or as monolayers in 35-mm diameter Petri dishes as described in previous publications (2, 8, 9). When cells were permeabilized, cultures in Petri dishes (1 × 10^6 cells/ml of medium/dish) were washed twice with a phosphate-free buffered salt solution (8) before permeabilizing the cells with streptolysin 0 (10) as described elsewhere. The permeabilized cells (which remained attached to the dish) were washed once more, and 1 ml of fresh buffered salt solution was added to the cultures.

Studies of the Metabolism of [3H]Inositol-labeled Inositol Phosphates in Cell Extracts—Cells were harvested from suspension culture by centrifugation. They were washed twice and then disrupted by sonication to yield an extract of 10^6 cells/ml (whole cell extract) (2). The medium (pH 7.2) used for these experiments contained (in

---

1 The abbreviations used are: HPLC, high performance liquid chromatography; EGTA, (ethylenediaminetetraacetic acid; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid. In the figures and table the designations of the inositol phosphates are indicated by the number (subscript) of phosphate (P) groups and their position on the inositol (I) ring, e.g. inositol 1,4,5-trisphosphate is I(1,4,5)P3. The asterisks indicate the position of radiolabel.

2 J. R. Cunha-Melo, M. A. Beaven, Biochim. Biophys. Acta, submitted for publication.
of water-saturated diethyl ether five times. The extract was then stored at -20 °C.

The d-myo-[2-3H; 3-32P]inositol 1,4,5-tetrakisphosphate was prepared by evaporating 2 μCi of d-myo-[2-3H]inositol 1,4,5-trisphosphate (1 Ci/mmol, Amersham Corp.) to dryness under a stream of nitrogen and then dissolving the labeled compound in a solution of 10 mM Tricine buffer (pH 8.0), 100 mM MgCl₂, 40 mM 2,3-bisphosphoglycerate, 6 mM ATP, and 32P (Sigma, 1 mCi/ml) in 1 ml of distilled water. To this solution were added 100 μCi of a solution of trichloroacetic acid (5%). An extract was prepared exactly as described above before storage at -20 °C.

Preparation of d-myo-[2-3H]inositol 1,3,4,5-Tetakisphosphate—This compound was prepared from d-myo-[2-3H]inositol 1,4,5-trisphosphate by phosphorylation of the 3'-position followed by dephosphorylation at the 5'-position by use of the homogenate of rat brain tissue (see above) which served as a source of both the kinase and phosphatase activities (3). Five μCi of d-myo-[2-3H]inositol 1,4,5-trisphosphate (1 Ci/mmol, Amersham Corp.) was evaporated to dryness and then dissolved in the solution of Tricine, 100 mM, and MgCl₂, 10 mM (800 μl) to which was added 100 μCi of a solution of ATP, 100 mM, and 50 μCi of 32P (5%). The mixture was incubated at 37 °C for 15 min. The reaction was terminated by addition of 500 μl of the solution of trichloroacetic acid (30%). An extract was prepared exactly as described above.

Purification of Radiolabeled Compounds—The extracts described above were applied individually to a Whatman Partisil SAX anion exchange column (25 x 0.46 cm) to which had been attached a "precolumn" to Whatman pellicular anion exchange resin. The radiolabeled inositol phosphates were eluted with ammonium phosphate (pH 3.8) at a flow rate of 1 ml/min (11). The radiolabeled inositol 1,4,5-trisphosphate was separated by the protocol described by Dean and Moyer (11). The radiolabeled inositol 1,3,4,5-tetrakisphosphate was separated on a column of 1.0 M ammonium formate buffer to elute inositol tetrakisphosphate. The fractions containing only the desired radiolabeled compound were pooled. The pooled fractions were eluted five times with distilled water before application to a column of 5 ml of AG 1-X8 (formate form, 100–200 mesh) anion exchange resin (Bio-Rad). Inorganic phosphate was eluted with 30 ml of a solution of 1 M ammonium formate to pH 7.0 to 3 M ammonium formate and 0.1 M formic acid. The inositol 1,4,5-trisphosphate was eluted with 20 ml of a solution of 1.0 M ammonium formate and 0.1 M formic acid. The inositol tetrakisphosphate was eluted with 30 ml of a solution of 1.4 M ammonium formate and 0.1 M formic acid. The fractions that contained the labeled compounds were pooled. The purified radiolabeled inositol compounds were dissolved in distilled water and stored at -20 °C.

Analysis of the purified labeled compounds by HPLC indicated a radiochemical purity of ~99% for d-myo-[2-3H; 4,5-32P]inositol 1,3,4,5-tetrakisphosphate and >95% for the d-myo-[2-3H; 3-32P]inositol 1,3,4,5-tetrakisphosphate. The latter compound contained trace amounts of [32P]inositol (1% of the [32P] label) and material that eluted between inositol 1,4,5-trisphosphate and inositol 1,3,4,5-tetrakisphosphate (~5% of the [32P] label).

RESULTS

Preliminary Studies with [3H]Inositol 1,3,4,5-Tetrakisphosphate and RBL-2H3 Cell Extracts—The incubation of extracts with whole cells of d-myo-[2-3H]inositol 1,3,4,5-tetrakisphosphate resulted in the early appearance of two major peaks, which were tentatively identified from their retention times as [2-3H]inositol 1,3,4-trisphosphate and inositol 1,4,5-bisphosphate. A small but distinct peak, which coeluted exactly with [4,5-32P]inositol 1,4,5-trisphosphate was also observed. Thereafter, two additional minor peaks of [2-3H]inositol bisphosphate and [2-3H]inositol monophosphate appeared (data not...
Identification of the Labeled Inositol Metabolites—A definitive identification of the labeled isomers was obtained from experiments with the two double-labeled preparations of inositol 1,3,4,5-tetrakisphosphate. The profiles of the products that were obtained with membrane fractions of RBL-2H3 cells are shown in Fig. 1 and the ratios of $^{32}$P to $^3$H in each inositol phosphate are indicated in Table I. For example, when D-myo-[2-$^3$H; 4,5-$^{32}$P]inositol 1,3,4,5-tetrakisphosphate was hydrolyzed (Fig. 1B), the identity of inositol 1,3,4-trisphosphate was confirmed, the ratio of $^{32}$P to $^3$H label remained unchanged when compared with the parent compound (Table I). In contrast, the putative inositol 1,4,5-trisphosphate and inositol 1,4-bisphosphate that were generated had, as expected, no $^{32}$P label. Two other inositol bisphosphates which eluted before and after the inositol 1,4-bisphosphate retained $^{32}$P label, although the high ratio of $^{32}$P to $^3$H label (in the parent compound) resulted in poorly defined peaks of $^3$H label. These two products, which were derived presumably from inositol 1,3,4-trisphosphate, were tentatively identified as inositol 1,3-bisphosphate and inositol 3,4-bisphosphate. Hydrolysis of D-myo-[2-$^3$H; 4,5-$^{32}$P]inositol 1,3,4,5-tetrakisphosphate (Fig. 1C) allowed unequivocal identification of inositol 1,4,5-trisphosphate because the ratio of $^{32}$P to $^3$H label was the same for both compounds (Table I), and the possibility of a 3,4,5-trisphosphate isomer was excluded by the loss of the 3-phosphate label in the previous experiment (i.e. Fig. 1B). The inositol 1,3,4-trisphosphate and inositol 1,4-bisphosphate retained, as expected, some $^{32}$P label, but examination of the data (Table I) suggested that $^{32}$P label was disproportionally distributed in the 5-phosphate and 4-phosphate position in the ratio 3.5 to 1.³ The inositol 3,4-bisphosphate retained a small but significant amount of $[^{32}P]4$-phosphate (or 5-phosphate) and this excluded the possibility of it being inositol 1,3-bisphosphate. The low counts, however, did not allow an accurate estimate of the ratio of $^{32}$P to $^3$H (Table I).

The two minor peaks of the [2-$^3$H]inositol bisphosphates that were generated in antigen-stimulated intact-cells (see Ref. 2) had retention times identical to those of the inositol 1,3-bisphosphate and inositol 3,4-bisphosphate described above. The same two peaks were obtained when permeabilized cells were incubated with [2-$^3$H]inositol 1,3,4,5-tetrakisphosphate but not with [2-$^3$H]inositol 1,4,5-trisphosphate (data not shown). As the peaks had similar retention times in all cases, we presumed that the inositol bisphosphates that are generated from either endogenous or exogenous substrates are the same.

Kinetics and Pathways of Metabolism of the Inositol Polyphosphates in RBL-2H3 Cell Extracts—The relative proportions of the 1,3,4-trisphosphate and the 1,4,5-trisphosphate isomers that were generated from the inositol tetrakisphosphate varied (e.g. Fig. 1B versus 1C). The studies, described below, indicated that such variation was attributable to kinetic considerations, and that factors such as the presence or absence of ATP or Ca$^{2+}$ influenced the time course and pathways of metabolism of the inositol polyphosphates.

As was observed in our preliminary experiments, incubation

³ Calculated from the ratio $[^{32}P]/[^3H]$ (Table I) as follows: $(0.09 - 0.02) + 0.02 = 3.5$. A similar disparity in the distribution of label in [4,5-$^{32}$P]inositol 1,4,5-trisphosphate (from which the radiolabeled inositol 1,3,4,5-tetrakisphosphate was made) has been reported by others (7, 20).
TABLE I

| Assumed structure | [3P] | [3H] | [3P]/[3H] |
|------------------|------|------|-----------|
| \( *I(1,3,4,5)P_4 \) | 46,750 | 8,146 | 5.7 (3.5)* |
| \( *I(1,4,5)P_3 \) | ND* | 649 | ND* |
| \( *I(1,3,4,5)P_4 \) | 15,306 | 2,313 | 6.5 (3.4) |
| \( *I(1,4)P_2 \) | ND | 197 | ND |
| \( *I(3,4)P_2 \) | 175 | 49 | 3.5 (3.5) |
| \( *I(1,3)P_2 \) | 234 | 40 | 5.9 (4.7) |
| \( *(1,3,4,5)P_4 \) | 1,203 | 12,855 | 0.09 (0.18) |
| \( *(1,4,5)P_3 \) | 215 | 2,321 | 0.09 (0.18) |
| \( *(1,3,4)P_3 \) | 209 | 8,634 | 0.02 (0.03) |
| \( *(1,4,5)P_3 \) | 33 | 1,407 | 0.02 (0.04) |
| \( *(3,4)P_2 \) | 13 | 222 | 0.06* |
| \( *I(1,3)P_2 \) | ND | 36 | ND |

* Values in parentheses were obtained in a second experiment.
* ND, no detectable peak.
* [3P] counts were too low for reliable measurement.

FIG. 2. Time course of metabolism of \( d \)-myo-[2-\( ^3H \)]inositol 1,3,4,5-tetrakisphosphate by membrane (Pellet) and soluble (Cytosol) fractions of RBL-2H3 cells. Upper panels show changes in concentration of ATP and ADP in the same experiments. The incubation mixture contained the labeled inositol phosphate (0.2 \( \mu \)M), cell extract (10^6 cells/ml), and ATP (2 \( \mu \)M) in a buffered potassium glutamate salt solution. Values are the mean \( \pm \) S.E. of 3-4 incubations. In this series of experiments, the predominant bisphosphate was inositol 1,4-bisphosphate (>98% of the bisphosphates in the membrane fraction: 90% in the cytosol fraction).

of \( [H] \)inositol 1,3,4,5-tetrakisphosphate with the membrane fraction resulted in the simultaneous formation of \( [H] \)inositol 1,3,4,5-tetrakisphosphate and \( [H] \)inositol 1,4,5-trisphosphate, although the appearance of the inositol 1,4,5-trisphosphate was transient because it was rapidly degraded to inositol 1,4-bisphosphate. In three series of experiments, one of which is shown in Fig. 2, the results were qualitatively similar but the proportion of the inositol 1,3,4,5-tetrakisphosphate converted to, and the time course of appearance and disappearance of, the inositol 1,4,5-trisphosphate varied. The experiment showing the most rapid changes (i.e. Fig. 2) indicated that inositol 1,4,5-trisphosphate reached maximal levels (>25% of total \( ^3H \) by 60 s. In the other experiments, maximal levels (30-35% of total \( ^3H \) of the inositol 1,4,5-trisphosphate occurred within 2-4 min and in one experiment the levels of inositol 1,4,5-trisphosphate exceeded those of inositol 1,3,4-trisphosphate at the early time points. On the assumption that inositol 1,4-bisphosphate was derived exclusively from inositol 1,4,5-trisphosphate (see below), the proportion of the inositol tetrakisphosphate converted to inositol 1,4,5-trisphosphate varied from 30 to 55% for all three experiments.

In contrast to the above, the amounts of inositol 1,4,5-trisphosphate generated in the cytosolic fraction of RBL-2H3 cells (e.g. Fig. 2) accounted for, at most, 7% (range 4-14%) of the total \( ^3H \) label in the three experiments. We did not determine whether these small amounts were generated by a soluble 3-phosphatase activity or by contaminating membrane enzyme activity.

The appearance of the inositol 1,4,5-trisphosphate occurred before the levels of ATP had declined substantially, although in the membrane fractions the levels of ATP declined by 50% over 5 min (upper panels, Fig. 2). Other experiments with whole cell extracts showed, however, that the rates of dephosphorylation of either the inositol tetrakisphosphate or its metabolites were reduced in the presence of ATP especially when ATP levels were maintained by an ATP-regenerating system. In the absence of ATP, the predominant pathway of dephosphorylation of inositol 1,3,4,5-tetrakisphosphate was through inositol 1,3,4-trisphosphate, inositol 1,3-bisphosphate, inositol 3,4-bisphosphate, inositol 1-monophosphate, and inositol. Small amounts of inositol 1,4-bisphosphate and inositol 1-monophosphate were also generated. In the presence of ATP, the predominant pathway was inositol 1,4,5-trisphosphate, inositol 1,4-bisphosphate, inositol 4-monophosphate, and inositol. Studies with the \( ^3H \)-labeled inositol trisphosphates showed, in addition, that in the absence of ATP the formation of inositol 1,3-bisphosphate from inositol 1,3,4-trisphosphate was a relatively minor pathway and that inositol 1,4-bisphosphate was largely, but not exclusively, degraded to inositol 4-monophosphate.

In the presence of ATP (2 \( \mu \)M) and the ATP-regenerating system, the dephosphorylation of inositol 1,4,5-trisphosphate was retarded and the dephosphorylation of inositol 1,3,4-trisphosphate was totally blocked. Instead, there was additional phosphorylation of inositol 1,4,5-trisphosphate (to form inositol 1,3,4,5-tetrakisphosphate), inositol 1,3,4-trisphosphate (to form an unidentified metabolite which was presumed to be an inositol tetrakisphosphate but which differed in retention time from inositol 1,3,4,5-tetrakisphosphate), and inositol 1,3,4,5-tetakisphosphate (to form inositol pentakisphosphate) (data not shown).

DISCUSSION

In this study we report the first example of the dephosphorylation of inositol 1,3,4,5-tetrakisphosphate via the removal of the 3'-phosphate to yield inositol 1,4,5-trisphosphate and of the 5'-phosphate to yield inositol 1,3,4-trisphosphate. The specific loss of the 32P label in the 3'-position in the inositol 1,4,5-trisphosphate peak excludes the misidentification of the product as either inositol 3,4,5-trisphosphate or inositol 1,3,5-trisphosphate. The 3'-phosphomonoesterase activity appears to be largely, possibly exclusively, a membrane enzyme, whereas the 5'-phosphomonoesterase activity is present in both the membrane and soluble fractions of the cell (Fig. 2). The absence of a 3'-kinase activity in the membrane fraction

* Either D- or L-myoinositol 1-monophosphate.
It should be noted that stimulation of RBL-2H3 cells requires the aggregation of plasma membrane receptors for immunoglobulin E (1) to induce hydrolysis of membrane inositol phospholipids, an increase in concentration of cytosol Ca\(^{2+}\), and the secretion of histamine (2, 5, 24). These reactions are dependent on the number of receptors clustered (8) and on the type of receptor cross-linking agent used (2). The mechanism of activation of RBL-2H3 cells (and mast cells) may therefore, differ in some respects from that in which cell activation requires the binding of a single hormone to a receptor. A unique property of the RBL-2H3 cell line is that, in the absence of external Ca\(^{2+}\), the stimulatory signals are weak or undetectable (2) and external Ca\(^{2+}\) is required for amplification of the stimulatory responses and for secretion to occur (2).

The question has been raised as to whether the formation of inositol 1,4,5,4,5-tetrakisphosphate by a Ca\(^{2+}\)-modulated 3'-kinase (5), which appears to be operative in RBL-2H3 cells, allows inactivation of inositol 1,4,5-trisphosphate or provides a second species of messenger. Recent studies by microinjection into sea urchin eggs suggests that inositol 1,4,5-tetrakisphosphate in coordination with the action of inositol 1,4,5-trisphosphate promotes influx of Ca\(^{2+}\) ions across the plasma membrane (25). If the same situation exists in RBL-2H3 cells, especially because of the substantial dependence of RBL-2H3 cells on external Ca\(^{2+}\), the 3'-kinase (3, 5) and the 3'-phosphomonoesterase (this paper) enzyme activities in RBL-2H3 cells could conceivably regulate the balance between both messengers. As both substances are substrates for the inositol 5'-phosphomonoesterase, whose activity is modulated by protein kinase C activity (26), one enzyme may inactivate simultaneously the two messengers. An interesting point emerging from the present work is this: if the presence of a 3'-phosphomonoesterase in membranes of RBL-2H3 cells is related to their reliance on extracellular Ca\(^{2+}\), is the enzyme less prominent in cell lines that can generate a Ca\(^{2+}\) signal by mobilization of intracellular Ca\(^{2+}\)? If so, information as to whether or not the enzyme is restricted to the plasma membrane would be instructive.

Since submission of this manuscript, Doughney and coworkers (27) have reviewed that human erythrocyte membranes metabolize inositol 1,3,4,5-tetrakisphosphate to inositol 1,4,5-trisphosphate, as well as inositol 1,3,4,5-trisphosphate, in the absence of Mg\(^{2+}\). These authors suggest, as we have done, that such a reaction in hormonally sensitive cells may provide a mechanism for the maintenance of constant concentrations of inositol 1,4,5-trisphosphate and inositol 1,3,4,5-tetrakisphosphate and may thus be important for stimulation of Ca\(^{2+}\) entry after Ca\(^{2+}\) mobilization.

REFERENCES

1. Metzger, H., Alcaraz, G., Hohman, R., Kinet, J. P., Pribhuda, V., and Quarto, R. (1986) Annu. Rev. Immunol. 4, 419-470
2. Cunha-Melo, J. R., Dean, N. M., Moyer, J. D., Mayeana, K., and Bean, M. A. (1987) J. Biol. Chem. 262, 11455-11463
3. Irvine, R. F., Letcher, A. J., Heqpol, J. F., and Berridge, M. J. (1986) Nature 320, 631-634
4. Storey, D. J., Shears, S. B., Kirk, C. J., and Michall, R. H. (1984) Nature 312, 374-376
5. Bieden, T. J., and Wollheim, C. B. (1986) J. Biol. Chem. 261, 11931-11934
6. Connolly, T. M., Bansal, S. V., Bross, T. E., Irvine, R. F., and Majerus, P. W. (1987) J. Biol. Chem. 262, 2414-2419
7. Shears, S. B., Storey, D. J., Morris, A. J., Cubitt, A. B., Parry, J. B., Michell, R. H., and Kirk, C. J. (1987) Biochem. J. 242, 395-402
8. Mayeana, K., Hohman, R. J., Metzger, H., and Bean, M. A. (1986) J. Biol. Chem. 261, 2583-2592


d) J. R. Cunha-Melo, N. M. Dean, H. Ali, and M. A. Bean, unpublished data
Metabolism of Inositol Tetrakisphosphate in RBL-2H3 Cells

9. Lo, T. N., Saul, W., and Beaven, M. A. (1987) J. Biol. Chem. 262, 4141–4145
10. Howell, T. W., and Gomperts, B. D. (1987) Biochim. Biophys. Acts 927, 177–183
11. Dean, N. M., and Moyer, J. D. (1987) Biochem. J. 242, 361–366
12. Dean, N. M., and Moyer, J. D. (1987) Biochem. J. 250, 493–500
13. Irvine, R. F., Letcher, A. J., Lander, D. J., and Berridge, M. J. (1986) Biochem. J. 240, 301–304
14. Shears, S. B., Parry, J. B., Tang, E. K. Y., Irvine, R. F., Michell, R. H. and Kirk, C. J. (1987) Biochem. Biophys. Res. Commun. 143, 363–359
15. Irvine, R. F., Letcher, A. J., Lander, D. J., Heslop, J. P., and Berridge, M. J. (1987) Biochem. Biophys. Res. Commun. 148, 199–205
16. Bansal, V. S., Inhorn, R. C., and Majerus, P. W. (1987) J. Biol. Chem. 262, 9444–9447
17. Balla, T., Baukal, A. J., Guillemette, G., and Catt, K. J. (1988) J. Biol. Chem. 263, 4083–4089
18. Balla, T., Guillemette, G., Baukal, A. J., and Catt, K. J. (1987) Biochem. Biophys. Res. Commun. 148, 199–205
19. Stephens, L., Hawkins, P. T., Carter, N., Chahwala, S. B., Morris, A. J. Whetton, A. D., and Downes, P. C. (1988) Biochem. J. 249, 271–282
20. Inhorn, R. C., Bansal, V. S., and Majerus, P. W. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 2170–2174
21. Hawkins, P. T., Stephens, L., and Downes, C. P. (1986) Biochem. J. 238, 507–516
22. Tenner, K. A., McKinney, J. S., and Putney, J. W., Jr. (1987) Biochem. J. 242, 797–802
23. Hansen, C. A., Mah, S., and Williamson, J. R. (1986) J. Biol. Chem. 261, 8100–8103
24. Pribluda, V., and Metzger, H. (1987) J. Biol. Chem. 262, 11449–11454
25. Irvine, R. F., and Moor, R. M. (1986) Biochem. J. 240, 917–920
26. Connolly, T. M., Lawing, W. J., and Majerus, P. W. (1986) Cell 46, 951–958
27. Doughney, C., McPherson, M. A., and Dormer, R. L. (1988) Biochem. J. 251, 927–929