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# STUDIES ON THE PROPERTIES OF A SOLUBLE PHOSPHATIDYLINOSITOL-PHOSPHODIESTERASE OF RABBIT IRIS SMOOTH MUSCLE

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## Summary

Some properties of the soluble phosphatidylinositol phosphodiesterase (monophosphatidylinositol inositolphosphohydrolase, EC 3.1.4.10) of rabbit iris smooth muscle are described. Studies on its subcellular distribution showed that in this tissue the phosphodiesterase is not exclusively cytosolic. Thus, under our experimental conditions about 58% of the enzyme activity was found in the soluble fraction and the remainder was particulate. When the latter was treated with deoxycholate about 59% of the enzyme activity, compared to 86% of that of ATPase, was still bound to the particulate fraction. The kinetic properties of the enzyme (30—50% $(NH_4)_2SO_4$ fraction) were examined. Maximum breakdown was 7.7 μmol/h per mg protein and occurred at pH 5.6. The products of [$^{14}$C]arachidonic acid-labelled phosphatidylinositol were 1,2-diacylglycerol and a mixture of 86% myoinositol 1-phosphate and 14% myoinositol 1,2-(cyclic)phosphate. The enzyme has an absolute requirement for $Ca^{2+}$. Addition of $Ba^{2+}$, $La^{3+}$, $Mg^{2+}$, $Mn^{2+}$, EGTA or EDTA at 0.05—5 mM concentrations; $Sr^{2+}$ at higher concentrations (greater than 0.25 mM) markedly inhibited the phosphodiesterase activity and this inhibition was completely reversed by $Ca^{2+}$. The enzyme is specific for the phosphoinositides.

## Introduction

The properties of phosphatidylinositol phosphodiesterase (monophosphatidylinositol inositolphosphohydrolase, EC 3.1.4.10), the enzyme that

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Abbreviations: triphosphoinositide, phosphatidyllinositol 4,5-bisphosphate; EGTA, ethyleneglycol bis(β-aminoethylether)-*N*,*N*′-tetraacetic acid.

catalyses the hydrolysis of phosphatidylinositol into 1,2-diacylglycerol and inositol 1-phosphate, have been investigated in a variety of tissues including brain [1—3], intestinal mucosa [4], lymphocytes [5,6] and *Bacillus cereus* [7]. This enzyme has been reported to occur in both particulate [2] and soluble [3] form in brain. An increased turnover of phosphatidylinositol in response to various stimuli has been reported in a variety of tissues and this increase appears to be the consequence of a stimulation of phosphatidylinositol breakdown into diacylglycerol and inositol monophosphate [8], presumably via the action of phosphatidylinositol phosphodiesterase.

Previously we reported that the neurotransmitters acetylcholine [9] and norepinephrine [10] at 0.05 mM and at short time intervals (less than 10 min) increase significantly the breakdown of triphosphoinositide and labelling of phosphatidic acid and phosphatidylinositol in rabbit iris which was prelabelled with $^{32}P_i$. These effects are dependent upon the presence of $Ca^{2+}$ in the incubation medium [11] and the enzyme that might be involved in this phenomenon appears to be triphosphoinositide phosphodiesterase (triphosphoinositide inositoltrisphosphohydrolase, EC 3.1.4.11) [12]. Recently we investigated the acetylcholine-stimulated breakdown of phosphoinositides in [$^3$H]inositol-labelled iris muscle and observed a marked increase in the production of inositol triphosphate and inositol monophosphate when $Ca^{2+}$ was added to the incubation medium [13]. The marked increase observed in the production of inositol monophosphate could also result from $Ca^{2+}$-activation of phosphatidylinositol phosphodiesterase, however, there was no concomitant decrease in the $^3$H-radioactivity of phosphatidylinositol. The increase in phosphatidylinositol labelling occurs despite the fact that the tissue no longer has access to [$^3$H]-inositol from the outside. This could suggest that in the presence of acetylcholine there is an increased hydrolysis of phosphatidylinositol followed by its resynthesis using an internal pool of [$^3$H]inositol which is not diluted out by the washing procedure. Furthermore there could be an increase in inositol monophosphate which could be derived from the hydrolysis of inositol triphosphate. In this connection it has recently been reported that the acetylcholine-stimulated increase in the labelling of phosphatidylinositol in synaptosomes is inhibited by EGTA and restored by the addition of $Ca^{2+}$ [14,15]. These findings are in contrast to those observed in other tissues, where acetylcholine-stimulated phosphatidylinositol turnover appears to be $Ca^{2+}$-independent [8]. These observations, coupled with the fact that there is little information about phosphatidylinositol phosphodiesterase in smooth muscle prompted the present study on the properties of this enzyme in the iris muscle.

## Materials and Methods

### *Materials*

Phosphatidylinositol (from bovine liver or porcine liver), monoacylglycerol and diacylglycerol were obtained from Avanti Biochemicals (Birmingham, AL) and from Serdary Research Laboratories (Lond, Canada). Myoinositol 2-monophosphate was purchased from Sigma Chemical Co. (St. Louis, MO).

Myoinositol 1-phosphate and myoinositol 1,2-(cyclic)phosphate were a gift from Dr. Frank Eisenberg, Jr., [1-$^{14}$C]arachidonic acid (40—60 Ci/mol) was

purchased from New England Nuclear, Boston, MA. [$^{14}$C]Arachidonic acid-labelled phosphatidylinositol was prepared by incubating 8 g rabbit iris in a modified Bradford medium [9] that contained 50 $\mu$Ci [$^{14}$C]arachidonic acid (bound to albumin) for 1 h at 37°C. The phospholipids were extracted as previously described [9] and [$^{14}$C]arachidonic acid-labelled phosphatidylinositol was separated and purified according to Sheltawy and Dawson [17] and Luthra and Sheltawy [18]. Diphosphoinositide and triphosphoinositide were prepared from bovine brain. A crude phosphoinositide fraction was prepared according to the method of Folch [19] and the polyphosphoinositides were isolated by means of DEAE-cellulose column chromatography (Whatman DE-52, microgranular), as described by Hendrickson and Ballou [20]. All other chemicals were reagent grade.

*Methods*

*Preparation of subcellular fractions from the rabbit iris muscle.* Methods of homogenization, subcellular fractionations and monitoring of the purity of the preparations by means of electron microscopy and enzyme markers were essentially as previously reported [12,16]. Briefly, the rabbit irises (approx. 8 g wet weight) were rinsed with ice-cold buffered saline (0.9% saline/10 mM Tris-HCl buffer, pH 7.4). The muscle strips were blotted and immersed into chilled 0.25 M sucrose (pH 7.4), then minced with scissors. The minced tissue was then suspended in 10 vols. buffered 0.25 M sucrose and homogenized for 4 × 30 s using a Super Dispax Tissumizer Model SDT-182 (Takmar Co.) at maximum speed. The homogenate was filtered through two layers of cheese cloth, then centrifuged at 600 × $g$ for 10 min in a refrigerated centrifuge. The nuclear pellet, which contained a considerable amount of cell debris was discarded. The supernatant was centrifuged at 10 000 × $g$ for 30 min and the mitochondrial fraction, which was contaminated with non-mitochondrial membraneous fragments, was retained as indicated. The supernatant obtained was then centrifuged at 110 000 × $g$ for 90 min to give a fairly purified microsomal fraction [16] and a soluble fraction. The microsomal fraction was retained as indicated and the soluble fraction was fractionated with $(NH_4)_2SO_4$ according to the procedure of Thompson [1]. The precipitate obtained between 30 and 50% saturation was dissolved in 5 mM Tris-maleate buffer (pH 5.6) and dialyzed overnight at 4°C against the same buffer. This dialyzed muscle extract (30—50% $(NH_4)_2SO_4$ fraction) was used in the following studies on the properties of phosphatidylinositol phosphodiesterase.

*Assay of phosphatidylinositol phosphodiesterase.* Phosphatidylinositol phosphodiesterase was assayed by a modification of the method of Thompson [1] in which the release of inositol phosphate was taken as a measure of the phosphodiesterase activity. The reaction mixture contained 50 mM Tris-maleate buffer (pH 5.6)/5 mM $CaCl_2$/1 mM phosphatidylinositol which was added directly as an aqueous emulsion, and 200 $\mu$g of the soluble enzyme protein in a final volume of 0.5 ml. After 5 min incubation at 37°C the reaction was stopped by the addition of 0.1 ml 5% (w/v) bovine serum albumin and 0.5 ml 10% (w/v) ice-cold $HClO_4$. The contents of the tubes were thoroughly mixed and kept in ice for 10 min then centrifuged for 20 min in a refrigerated centrifuge and the clear supernatant analyzed for organic phosphate according to the

method of Bartlett [21]. Appropriate incubation controls containing enzyme alone and substrate alone were carried through the same procedure.

*Isolation of the products of enzymic hydrolysis.* In larger-scale incubations, 1.5 μmol [$^{14}$C]arachidonic acid-labelled phosphatidylinositol (5200 cpm) was incubated in a final volume of 1 ml containing 50 mM Tris-maleate buffer (pH 5.6)/5 mM $CaCl_2$/1 mg enzyme protein. Incubations were for 1 h at 37°C. The reaction was terminated by addition of 2 ml $CHCl_3/CH_3OH/HCl$ (200 : 200 : 1, v/v). After centrifugation the upper and lower phases were analyzed for the water and organic-soluble products respectively. The water-soluble hydrolysis products were analyzed by ascending paper chromatography on Whatman 3 MM paper using pure myoinositol phosphates as standards. Ethanol/13.5 M ammonia (3 : 2, v/v) was used as solvent for development [7]. The spots were visualized with Hanes-Isherwood's reagent [22], and those corresponding to inositol 1-phosphate and inositol 1,2-(cyclic)phosphate were cut out and their phosphate contents analyzed. The $CHCl_3/CH_3OH$ (2 : 1, v/v) soluble lipid products were analyzed by paper chromatography on Whatman SG-81 silica gel loaded paper using monoacylglycerol, diacylglycerol and arachidonic acid as standards. Petroleum ether/diethyl ether/acetic acid (45 : 10 : 1, v/v) was used as solvent for development. The spots were visualized with iodine vapour and those corresponding to phosphatidylinositol, diacylglycerol and arachidonic acid were cut out and counted in a Beckman liquid scintillation counter.

$Mg^{2+}$-ATPase was assayed as described previously [23]. Protein was estimated by the method of Lowry et al. [24] using bovine serum albumin as standard.

## Results

*Distribution of phosphotidylinositol phosphodiesterase and $Mg^{2+}$-ATPase in the rabbit iris muscle*

Phosphatidylinositol phosphodiesterase has been reported to occur in both particulate [2] and soluble [3] form in brain, however, its precise location is not settled yet [3]. Since the enzyme ATPase is an integral membrane protein [25] it was of interest to compare its distribution and the effect of sodium deoxycholate on its release with those of the phosphodiesterase. As can be seen from Table I, about 58% of the phosphodiesterase and 17% of the ATPase activities were recovered in the soluble fraction. Most of the phosphodiesterase activity in the soluble fraction was found in the 30—50% $(NH_4)_2SO_4$ fraction. When the particulate fraction was treated with deoxycholate about 41% of the phosphodiesterase activity and 14% of the ATPase activity were released (Table I). Comparative studies conducted on the properties of the phosphodiesterase, e.g. pH optima and the effect of substrate concentration, in the mitochondrial-microsomal and soluble fractions (30—50% $(NH_4)_2SO_4$ fraction) revealed little difference between them (data not shown).

*Products of the phosphodiesterase action on phosphatidylinositol*

When the phosphodiesterase was incubated with [$^{14}$C]arachidonic acid-labelled phosphatidylinositol, the reaction products recovered in the water-soluble fraction were identified as inositol monophosphate and inositol

TABLE I

SUBCELLULAR DISTRIBUTION OF PHOSPHATIDYLINOSITOL PHOSPHODIESTERASE AND $Mg^{2+}$-ATPase AND THE EFFECT OF DEOXYCHOLATE ON THEIR RELEASE FROM THE PARTICULATE FRACTION

Homogenization of the iris muscle and preparation of subcellular fractions were the same as described under Methods. The enzyme assay consisted of 50 mM Tris-maleate buffer (pH 5.6)/5 mM $CaCl_2$/1 mM phosphatidylinositol/200 μg of protein in a final volume of 0.5 ml. After incubation for 5 min at 37°C, the inositolphosphate released during the reaction was determined as organic phosphate. $Mg^{2+}$-ATPase was assayed as described previously [23].

| Fraction | Protein (percent of homogenate) | Phosphatidylinositol phosphodiesterase | | $Mg^{2+}$-ATPase | |
|---|---|---|---|---|---|
| | | Specific activity * | Percent of total activity | Specific activity ** | Percent of total activity |
| Homogenate | 100 | 1.4 | 100 | 4.2 | 100 |
| 110 000 × *g* supernatant (soluble) | 44 | 1.7 | 58 | 1.6 | 17.2 |
| 110 000 × *g* precipitate (particulate) | 56 | 1.3 | 39 | 7.1 | 80 |
| 30—50% $(NH_4)_2SO_4$ precipitate from soluble fraction | 26 | 2.7 | 27 | 1.4 | 4.5 |
| Precipitate from deoxycholate-treated particulate fraction *** | 44 | 1.0 | 23 | 6.8 | 69 |
| 30—50% $(NH_4)_2SO_4$ precipitate from supernatant of deoxycholate-treated particulate fraction | 5 | 3.6 | 8.5 | 2.7 | 2.1 |

* μmol organic phosphate released per mg protein per h.

** μmol $P_i$ liberated per mg protein per h.

*** The particulate fraction was homogenized in 50 mM Tris-maleate buffer (pH 5.6) containing 1 mg deoxycholate per ml by a glass homogenizer. The precipitate was suspended in 5 mM buffer and dialyzed overnight against the same buffer.

1,2-(cyclic)phosphate and in the $CHCl_3/CH_3OH$ (2 : 1, v/v) fraction the product was identified as 1,2-diacylglycerol. On the basis of the recoveries in the water-soluble and chloroform-soluble fractions, one can conclude that about 34 and 29% of phosphatidylinositol was hydrolyzed by the enzyme, respectively (Table II). About 86% of the liberated water-soluble inositol phosphates were recovered as myoinositol 1-phosphate and about 14% as myo-

TABLE II

PRODUCTS OF HYDROLYSIS OF [$^{14}$C]ARACHIDONIC ACID-LABELLED PHOSPHATIDYLINOSITOL BY PHOSPHATIDYLINOSITOL PHOSPHODIESTERASE

[$^{14}$C]Arachidonic acid-labelled phosphatidylinositol (1.5 μmol, 5200 cpm) was incubated for 1 h at 37°C in 1 ml of 50 mM Tris-maleate buffer (pH 5.6) containing 5 mM $CaCl_2$ and 1 mg of the soluble enzyme (the 30—50% $(NH_4)_2SO_4$ fraction).

| Expt. no. | Water-soluble products (μmol organic phosphate) | | Chloroform/methanol-soluble products (cpm) | | |
|---|---|---|---|---|---|
| | Inositol phosphate | Inositol 1,2-(cyclic)-phosphate | Phosphatidyl inositol | 1,2-Diacyl-glycerol | Arachidonic acid |
| 1 | 0.433 | 0.069 | 3500 | 1389 | 8 |
| 2 | 0.450 | 0.081 | 3290 | 1578 | 10 |

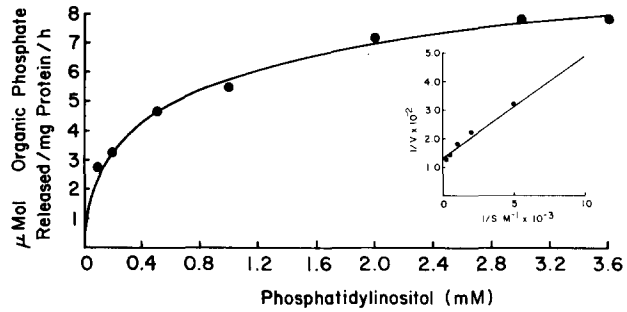


Fig. 1. Phosphatidylinositol phosphodiesterase activity as a function of substrate concentration.

inositol 1,2-(cyclic)phosphate. In the chloroform fraction almost all of the liberated $^{14}$C-radioactivity was found in 1,2-diacylglycerol (Table II). Clearly, these data suggest that the major activity was due to a phosphodiesterase attacking phosphatidylinositol.

*Properties of phosphatidylinositol phosphodiesterase*

*Effect of substrate.* The effect of various concentrations of phosphatidylinositol on the enzyme activity was tested under standard conditions. The plot of phosphatidylinositol phosphodiesterase as a function of substrate concentration is shown in Fig. 1. The activity of the enzyme increased as a function of substrate concentration and reached a maximum at about 3 mM. There was relatively little increase in rate at substrate concentrations above 3 mM. A Lineweaver-Burk plot of the data in Fig. 1 gave a $K_m$ value for the phosphodiesterase of $2.7 \cdot 10^{-4}$ M and a $V$ value of 7.7 μmol organic phosphate released/mg per h.

*Effect of pH.* The change in activity with the pH of the reaction mixture is

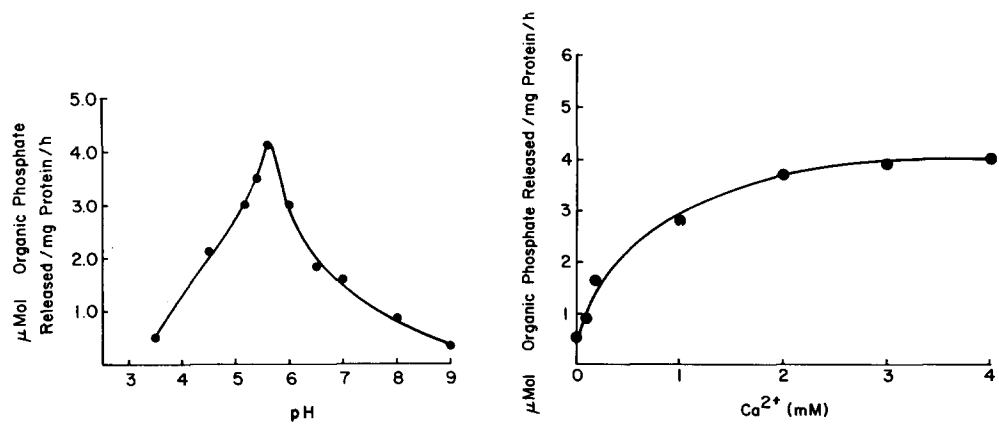


Fig. 2. pH dependence of soluble phosphatidylinositol phosphodiesterase. Phosphatidylinositol was incubated with the phosphodiesterase. Each pH was adjusted using Tris-maleate/NaOH buffer.

Fig. 3. Effects of $Ca^{2+}$ concentration on phosphatidylinositol phosphodiesterase.

TABLE III

EFFECTS OF DIVALENT CATIONS ON THE ACTIVITY OF PHOSPHATIDYLINOSITOL PHOSPHODIESTERASE

| Additions | Enzyme activity (percent of control) at cations concentration (mM) | | | | |
|---|---|---|---|---|---|
| | 0.05 | 0.25 | 1.0 | 2.5 | 5.0 |
| $SrCl_2$ | 113 | 128 | 54 (120; 217; 273) * | 22 | 12 |
| $BaCl_2$ | 89 | 83 | 81 (146; 240; 295) | 26 | 20 |
| $LaCl_3$ | 96 | 41 | 6 (212; 244; 311) | 3 | 1 |
| $MgCl_2$ | 68 | 53 | 35 (77; 183; 229) | 28 | 23 |
| $MnCl_2$ | 70 | 37 | 27 (119; 185; 256) | 15 | 12 |

* In the presence of 0.25 mM, 1.0 mM and 2.5 mM $Ca^{2+}$, respectively.

shown in Fig. 2. The optimum pH value was 5.6. A similar pH optimum was observed with the soluble enzyme obtained from the deoxycholate-treated particulate fraction (data not shown).

*Effect of enzyme concentration.* Under the standard assay conditions, hydrolysis of phosphatidylinositol by the phosphodiesterase was a linear function of the amount of protein added up to 250 μg.

*Time course of the hydrolysis of phosphatidylinositol.* At optimum concentrations of phosphatidylinositol and the enzyme protein the rate of reaction was linear up to 10 min then levelled off with time of incubation.

*Effects of $Ca^{2+}$ and other divalent and monovalent cations.* Low concentrations of $Ca^{2+}$ (100 μM) increased the activity of the phosphodiesterase by 60% and at 1 mM $Ca^{2+}$ the activity of this enzyme increased by 6-fold (Fig. 3). Further increase in $Ca^{2+}$ concentration resulted in a gradual increase in the phosphodiesterase activity and this levelled off at 4 mM concentration of the cation. The requirement for $Ca^{2+}$ is specific. Thus when $Ca^{2+}$ was replaced by various concentrations of $Ba^{2+}$, $La^{3+}$, $Mg^{2+}$ or $Mn^{2+}$ there was a steady decrease in the phosphodiesterase activity with increase in the cation concentration (Table III). Of the cations investigated only $Sr^{2+}$, at low concentrations (less than 0.25 mM), exerted a slight stimulatory effect on the phosphodiesterase, but at higher concentrations (greater than 0.25 mM) it inhibited the enzyme

TABLE IV

SUBSTRATE SPECIFICITY OF PHOSPHATIDYLINOSITOL PHOSPHODIESTERASE

The assay for phosphatidylinositol phosphodiesterase was conducted at pH 5.6 while the assays for the other phospholipids were carried out at pH 7.2

| Phospholipid | Phosphodiesterase | |
|---|---|---|
| | Specific activity * | % |
| Phosphatidylinositol | 4.3 | 100 |
| Diphosphoinositide | 1.5 | 34.9 |
| Triphosphoinositide | 1.6 | 37 |
| Phosphatidylcholine | 0 | 0 |
| Phosphatidylethanolamine | 0 | 0 |

* μmol organic phosphate released per mg protein per h.

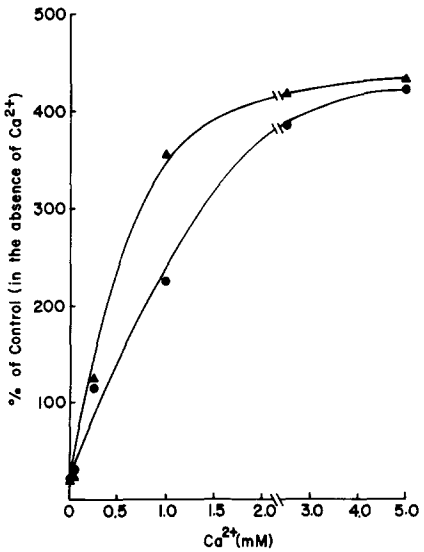


Fig. 4. Effects of $Ca^{2+}$ concentration on phosphatidylinositol phosphodiesterase in the presence of 0.25 mM EDTA (▲———▲) and 1.0 mM EGTA (●———●).

activity by more than 85% of that of the control. The inhibitory influence of these cations was completely reversed by $Ca^{2+}$ (Table III).At concentrations ranging between 5—100 mM the monovalent cations $Na^+$ and $K^+$ had no effect on the phosphodiesterase activity (data not shown).

*The influence of EDTA and EGTA on the phosphodiesterase activity and its reversal by $Ca^{2+}$.* In preliminary studies on the influence of EDTA and EGTA on the phosphodiesterase activity, it was found that the former chelating agent is a much more potent inhibitor of the enzyme activity than the latter. Thus it was found that about 80% of the phosphodiesterase activity was inhibited by 1 mM EGTA compared to 0.25 mM of EDTA (Fig. 4). The inhibitory effects of the chelating agents were completely reversed by $Ca^{2+}$ (Fig. 4).

*Substrate specificity.* As can be seen from Table IV, the enzyme preparation contained in addition to phosphatidylinositol phosphodiesterase, diphosphoinositide and triphosphoinositide phosphodiesterase activities. No release of organic phosphates was observed when phosphatidylcholine or phosphatidylethanolamine were used as substrates.

## Discussion

The data presented on the nature of the enzyme action on phosphatidylinositol clearly indicate that the rabbit iris muscle contains a soluble enzyme which cleaves the phospholipid between the phosphate group and the glycerol backbone, with release of 1,2-diacylglycerol and a mixture of myoinositol 1-phosphate and myoinositol 1,2-(cyclic)phosphate (Table II). The water-soluble inositol phosphates in the present study were about 14% myoinositol 1,2-(cyclic)phosphate, the remainder being myoinositol 1-phosphate. This is in contrast to the thyroid [26] and brain [3] where the myoinositol 1,2-(cyclic)-

phosphate was 62—65% and 55% of the total water-soluble inositol phosphates, respectively. The results on the subcellular distribution of the phosphodiesterase in the iris muscle (Table I) indicate that the enzyme is partly soluble (58%) and partly associated with membranes. This conclusion is based on the following findings: a. Under the present experimental conditions in which the smooth muscle was vigorously homogenized about 40% of the total phosphodiesterase activity was recovered in the particulate fraction. b. When the particulate fraction was treated with sodium deoxycholate about 59% of the phosphodiesterase activity, compared to 86% of that of the ATPase activity, remained bound to the particulate fraction. c. The finding that the 30—50% $(NH_4)_2SO_4$ fraction of the phosphodiesterase activity released by deoxycholate exhibited the same pH optimum (5.6) as that of the soluble enzyme suggests that we are dealing with the same protein. d. The observation that after the detergent treatment considerably less phosphodiesterase than ATPase activity remained bound to the particulate fraction, suggests that the former enzyme could only be loosely bound to the membranes. Irvine and Dawson [3] have presented good experimental evidence which suggests that in brain the membrane-bound enzyme reported to be membraneous by Lapetina and Michell [2] can be accounted for by soluble enzyme entrapped in the particulate fraction. In view of the data presented and taking into consideration the vast differences between the two tissues as well as the methodology employed in their homogenization it can be concluded that in the iris muscle the phosphodiesterase is not exclusively cytosolic.

The apparent $K_m$ value at pH 5.6 for the phosphodiesterase was found to be $2.7 \cdot 10^{-4}$ M (Fig. 1). $K_m$ value for the phosphodiesterase in the soluble fraction of rat liver was reported to be $3.3 \cdot 10^{-4}$ M [27]. The pH optimum [5.6] of the muscle phosphodiesterase is similar to the soluble enzymes in other tissues which are most active at pH values between 5 and 6.

The data presented on the effects of cations on the iris muscle phosphodiesterase indicate that this enzyme has an absolute requirement for $Ca^{2+}$ (Figs. 3, 4 and Table III). Thus both EDTA and EGTA abolished its activity and this inhibition was reversed by the addition of $Ca^{2+}$ (Fig. 4). The requirement for $Ca^{2+}$ is also specific. Thus, with the exception of $Sr^{2+}$ which was weakly effective at low, but inhibitory at higher, concentrations, $Ba^{2+}$, $La^{3+}$, $Mg^{2+}$ and $Mn^{2+}$ inhibited significantly at all concentrations investigated (0.5—5 mM). Again the inhibitory effects of all of these cations were reversed upon the addition of $Ca^{2+}$ (Table III). The finding that $Sr^{2+}$ can partially replace $Ca^{2+}$ is not surprising since this cation can replace $Ca^{2+}$ in several biological systems inclusing synaptic transmission [28]. In the presence of EDTA (0.25 mM) $Ca^{2+}$ at 0.25 mM increased the phosphodiesterase activity by 8-fold and at 2.5 mM it increased it by 23-fold. This selectivity suggests that $Ca^{2+}$ may have a more specific role in the reaction of this enzyme with its substrate [1]. Atherton and Hawthorne [4] suggested that the $Ca^{2+}$-activation is either due to the production of a suitable surface charge on the substrate particles or in others the cation may play a specific role as a coenzyme. In contrast to the brain enzyme [1] the monovalent cations $Na^+$ and $K^+$ had no effect on the iris muscle phosphodiesterase.

The present experiments indicate that in this soluble enzyme preparation the

polyphosphoinosities were hydrolyzed at less than half the rate of phosphatidylinositol breakdown, however, it must be emphasized that this calculation is derived from two different assay systems (Table IV). The finding that the soluble phosphatidylinositol phosphodiesterase has an optimum pH at 5.6 (Fig. 2), compared to pH of 7.2 for the microsomal triphosphoinositide phosphodiesterase [12], could suggest that we are dealing with two separate enzymes.

The possible role of phosphodiesterases in the reactions that lead to enhanced phosphatidylinositol labelling in a variety of tissues including the iris muscle has been discussed by several investigators (see Introduction). The present findings on the phosphodiesterase of the iris muscle add further support to the developing concept that the increase in phosphatidylinositol labelling in response to receptor activation could also be mediated via $Ca^{2+}$-stimulation of phosphoinositide phosphodiesterase [29].

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