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PHOSPHATIDYLINOSITOL KINASE OF BOVINE ADRENAL CHROMAFFIN GRANULES

MODULATION BY HYDROPHILIC AND AMPHIPHILIC CATIONS

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Abstract—The effects of hydrophilic and amphiphilic cations on the activity of phosphatidylinositol (PI) kinase (EC 2.7.1.67) of chromaffin granule ghosts were investigated. The cations studied can be divided into two groups, i.e. (i) compounds with a biphasic response (stimulation and inhibition), and (ii) those with a selective stimulatory effect on the enzyme activity. The cationic amphiphile trifluoperazine belongs to the first group, and stimulated the enzyme activity, maximal at 80 μM (2-fold), with a progressive inhibition at higher concentrations. This biphasic response was shared by a number of structurally related cationic amphiphiles, i.e. the tricyclic antidepressants, imipramine and desipramine, the phenothiazine, chlorpromazine, the miconazole derivative, calmidazolium, the β-adrenergic agonist, propranolol, compound 48/80, as well as by the hydrophilic cations neomycin and poly-L-lysine. On the other hand, a pure stimulatory effect was observed with the amphiphilic polypeptide mastoparan and the polycationic compound spermidine, whereas $ACTH_{1-39}$ and $ACTH_{1-24}$ (peptides structurally related to mastoparan) revealed a slight inhibitory effect. We conclude that all the cations tested including Mg^{2+}, stimulate PI kinase activity rather unspecifically by binding of the positively charged groups to a membrane component, probably the PI kinase itself. This site is different from that mediating the specific inhibition by calcium (Husebye ES and Flatmark T, *Biochim Biophys Acta* **968**: 261–265, 1988). The inhibitory effect of cationic amphiphiles is correlated to their lipid solubility, and represents a perturbation of the membrane structure, but not a solubilization of enzyme or phosphoinositide from the membrane. The inhibitory effect of hydrophilic cations is due to complexation of ATP.

Polyphosphoinositides play a central role in the cellular signal transduction of a wide variety of neurotransmitters, hormones and growth factors [1]. For instance, the secretion of catecholamines from adrenal chromaffin cells in response to stimulation by acetylcholine occurs by exocytosis [2], and the turnover of phosphoinositides has been shown to be involved in this process [3, 4].

The turnover of the polyphosphoinositides phosphatidylinositol 4-phosphate (PIP)* and phosphatidylinositol 4,5-bisphosphate (PIP_2) leads to production of several second messengers, i.e. (i) diacylglycerol, known to activate protein kinase C [5], (ii) arachidonic acid, an ionophore [6, 7] and precursor of prostanoids, and (iii) different inositol phosphates, which regulate the influx of calcium from internal stores (inositol 1,4,5-trisphosphate) [8]. Since both PIP and PIP_2 are precursors of second messengers, it is important to understand how their turnover is regulated and modulated pharmacologically. While the breakdown of polyphosphoinositides by different hydrolytic enzymes has been studied extensively, little is known about the regulation of the enzymes involved in the biosynthesis of these phospholipids.

In the present report we have addressed this question by studying the effects of hydrophilic and amphiphilic cations on the activity of phosphatidylinositol (PI) kinase (EC 2.7.1.67), a putative rate-limiting enzyme in the synthesis of polyphosphoinositides [9]. Previous reports have shown that cationic amphiphiles increase the *de novo* synthesis of PI (for review, see Refs 10 and 11), while both cationic amphiphiles and polyamines stimulate *de novo* synthesis [12–18] and turnover [17] of PIP and PIP_2. We have extended the latter studies and shown that a wide variety of cations modulate PI kinase activity, providing new insight into the regulation and pharmacological modulation of this enzyme.

MATERIALS AND METHODS

Materials. [γ-^{32}P]ATP (PB.10168, specific activity, 3000 Ci/mmol) was obtained from Amersham (Amersham, Bucks., U.K.). Phosphatidylinositol 4-phosphate, chlorpromazine, trifluoperazine, propranolol, imipramine, spermidine, putrescine, compound 48/80, poly-L-lysine (M_r 41,000 and 3700), $ACTH_{1-24}$ (human synthetic), $ACTH_{1-39}$ (human synthetic), diethylstilbestrol, and calmodulin from bovine brain were obtained from the Sigma Chemical Company (St Louis, MO). Calmidazolium was obtained from Calbiochem (San Diego, CA), nor-

* Abbreviations used: PI, phosphatidylinositol; PIP, phosphatidylinositol 4-phosphate; PI kinase, phosphatidylinositol kinase; Hepes, 4-(2-hydroxyethyl)-1-piperazine-2-ethanesulfonic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*, *N*-tetraacetic acid; ACTH, adrenocorticotropic hormone; BSA, bovine serum albumine; TLC, thin-layer chromatography.

adrenaline from Koch-Light Laboratories Ltd (Colnbrook, Berks., U.K.), mastoparan from Peninsula Laboratories Inc. (Belmont, CA), phosphatidylinositol from soybean was obtained from Pharmacia (Uppsala, Sweden), neomycin sulfate from Roussel Laboratories Ltd. (London, U.K.) and essentially fatty acid free bovine serum albumin was from Miles Biochemicals (Elkhart, IN). Desipramine was a gift from Ciba-Geigy AG (Basel, Switzerland).

Preparation of chromaffin granule ghosts. Highly purified chromaffin granule ghosts were prepared from freshly collected bovine adrenal glands [19]. 7.5 mM Pipes buffer with 0.2 mM EGTA, pH 7.5 was used as the medium for lysis of the chromaffin granules [19].

Assay of PI kinase activity with intrinsic PI as the substrate. The standard assay was performed at 25° for 1 min in a medium (0.3 ml) containing chromaffin granule ghosts (approx. 50 μg of protein), 30 mM Hepes pH 7.0, 0.1 mM EGTA, 1 mM $MgCl_2$, and 0.1 mM [γ-^{32}P]ATP. The PI kinase activity was linear in the time and protein concentration range used. The drugs and peptides were added in stock solutions dissolved in water with the exception of calmidazolium and diethylstilbestrol which were dissolved in ethanol. The reaction mixture containing the granule ghosts was preincubated at 25° for 90 sec before the reaction was started by adding ATP. After 60 sec the reaction was stopped by transferring an aliquot (0.24 ml) into ice-cold chloroform/methanol/12 M HCl (20:40:1, v/v) [20].

Assay of Triton X-100-solubilized PI kinase using purified PI as the substrate. Membrane proteins were solubilized by incubating chromaffin granule ghosts with 0.2–1% (w/v) Triton X-100 in 7.5 mM Hepes pH 7.0 for 15 min at 0°, before centrifugation at 213,000 *g* for 10 min. The supernatant was used as the source of PI kinase. The PI substrate was dispersed in 7.5 mM Hepes pH 7.0 and sonicated for 15 min in a G112-SP-T s< icator (600 V, 80 cycles) from Laboratory Supplies Co. Inc. (Hickersville, NY). The activity was assayed as described above, using 0.1 mg PI/ml as the substrate.

Stability of [^{32}P]*PIP in chromaffin granule ghosts.* PIP was labelled with ^{32}P by incubating chromaffin granule ghosts for 20 min at the conditions described above. The ghosts were then rapidly gel-filtered at 4° using a 1.5 × 15 cm column of Sephadex G-50, medium (Pharmacia, Uppsala, Sweden) equilibrated with 7.5 mM Hepes buffer, pH 7.0. The gel-filtered ghosts (containing about 700 cpm/μg of protein) were then incubated for 9 min in a medium (1.5 ml) containing 30 mM Hepes, 0.1 mM EGTA; pH 7.0, with and without trifluoperazine. Subsequent extraction of phospholipids and assay of [^{32}P]PIP was performed as described below.

Extraction and isolation of phospholipids. Phospholipids were extracted with ice-cold chloroform/methanol/12 M HCl (20:40:1, v/v) [20]. The lower phase was removed, dried under streaming nitrogen, resuspended in chloroform/methanol (2:1, v/v), and immediately applied on to DC-Alufolien Kieselgel 60 plates (Merck, Darmstadt, F.R.G.) pretreated with 1% (w/v) potassium oxalate by chromatography. The plates were run in a solvent containing chloroform/methanol/20% methylamine (60:36:20, v/v) as described [21]. After radioautography, the ^{32}P-containing spots were identified by radioactive standards [22] kindly provided by Prof. H. Holmsen at this department. The radioactive spots were scraped from the plates and counted for radioactivity in a liquid-scintillation spectrometer (Packard Tri-Carb 460 CD) with 4 ml Opti-Fluor (Packard Instrument International, Zürich, Switzerland).

Other methods. Protein was determined by the method of Bradford [23] using bovine serum albumin as the standard.

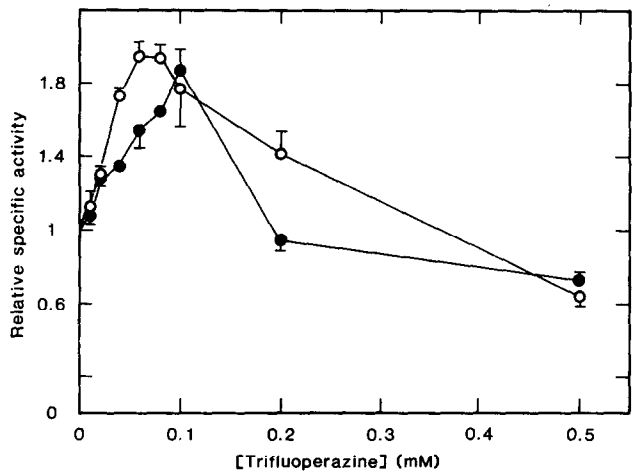

Fig. 1. The effect of trifluoperazine on PI kinase activity. Incubations were performed at 25° for 1 min in a reaction mixture (0.3 ml) containing 30 mM Hepes, 0.1 mM EGTA, 1 mM $MgCl_2$, 0.1 mM [γ-^{32}P]ATP; pH 7.0. (○) endogenous substrate; (●) exogenous substrate at 0.1 mg PI/ml. The results (means ± SD, N = 3) are given as relative specific activities. The specific activity of the controls were 1.04 ± 0.015 and 0.50 ± 0.030 nmol PIP/mg protein per min (means ± SD, N = 3) for endogenous and exogenous substrate, respectively.

Table 1. The effect of trifluoperazine on PI kinase activity at different concentrations of $MgCl_2$

$MgCl_2$	Control	0.08 mM TFP	0.5 mM TFP
(mM)	Relative specific activity		
1	1.0	1.88 ± 0.07	0.57 ± 0.01
5	1.0	1.18 ± 0.08	0.69 ± 0.01
20	1.0	1.00 ± 0.01	0.69 ± 0.04

Incubations were performed at 25° for 1 min in a reaction mixture (0.3 ml) containing granule membranes, 30 mM Hepes, 0.1 mM EGTA, $MgCl_2$, 0.1 mM [γ-^{32}P]ATP; pH 7.0 and 0.08 or 0.5 mM trifluoperazine (TFP). The results (means ± SD, $N = 3$) are given as relative specific activities. The specific activity of the controls were 1.59 ± 0.017, 3.30 ± 0.078 and 4.34 ± 0.025 nmol PIP/mg protein per min (means ± SD, $N = 3$) for 1, 5 and 20 mM $MgCl_2$, respectively.

RESULTS

Effect of trifluoperazine on PI kinase activity

At the standard incubation condition (1 mM $MgCl_2$ and pH 7.0), trifluoperazine revealed a biphasic effect on PI kinase activity (Fig. 1). This effect was observed with membrane-bound enzyme and intrinsic PI, as well as with solubilized enzyme and exogenously added PI as the substrate (Fig. 1). A stimulation was observed at 10 μM, reaching a maximum at 80 μM. The stimulatory effect of 80 μM trifluoperazine was highly dependent on the concentration of $MgCl_2$ (Table 1), and maximal at pH 7.5 in the presence of 1 or 5 mM Mg^{2+} (data not shown). Similar effects of Mg^{2+} and pH was observed with chlorpromazine (data not shown). Preincubation periods longer than the regular 90 sec, i.e. 270 and 570 sec, did not change the effect of either 0.1 or 0.5 mM trifluoperazine. This finding indicates a rather rapid interaction of the phenothiazine with the granule membrane.

The inhibitory effect of high concentrations of trifluoperazine (0.5 mM) (Fig. 1) was relatively insensitive to the concentration of $MgCl_2$ (Table 1), and not influenced by changes in the concentration of ATP (0.1–0.4 mM). Diethylstilbestrol, a non-charged bicyclic hydrophobic compound, was found to have a pure inhibitory effect on PI kinase activity (Fig. 2).

The possibility that trifluoperazine inhibited PI kinase activity by solubilizing membrane components was also investigated. The solubilization of proteins was assayed by incubating chromaffin granule membranes (BSA washed) with 0.5 mM trifluoperazine for 15 min at 0°. 8.2 and 10.4% of the proteins were released in the control and trifluoperazine incubation, respectively. Assay of PI kinase activity (0.1 mg PI/ml) in the supernatant after centrifugation (213,000 g for 15 min) gave the numbers 0.93 ± 0.04 and 0.42 ± 0.01 pmol PIP/min (means ± SD, N = 3) for the control and trifluoperazine incubations, respectively. On the other hand, solubilization with 0.2% (w/v) Triton X-100 under identical conditions released 83% of the protein and resulted in a large increase in PI kinase activity in the supernatant (51.4 ± 0.50 pmol/min PIP, mean ± SD, N = 3).

Solubilization of phospholipids was assayed on the basis of the release of intrinsic [^{32}P]PIP from chromaffin granule membranes labelled with [γ-^{32}P]ATP and gelfiltered (see Materials and Methods). Incubation and centrifugation were performed as described above for the solubilization of membrane proteins. Neither 0.5 mM trifluoperazine nor 0.5 mM diethylstilbestrol released significant

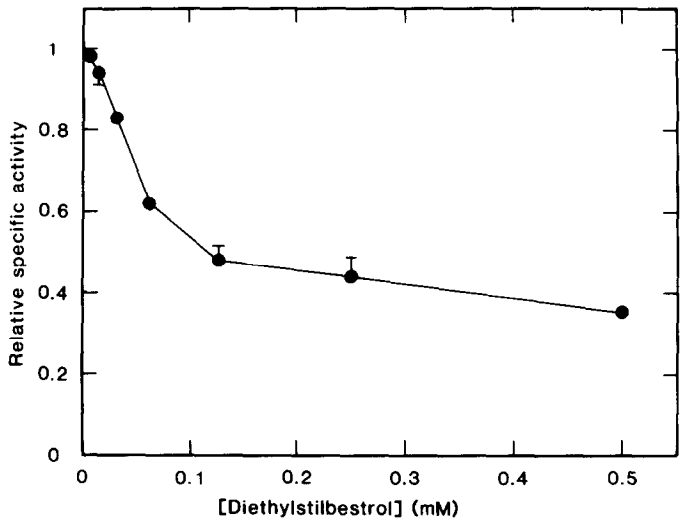

Fig. 2. The effect of diethylstilbestrol on PI kinase activity. Incubations were performed at 25° for 1 min in a reaction mixture (0.3 ml) containing granule ghosts (132 μg/ml), 30 mM Hepes, 0.1 mM EGTA, 5 mM $MgCl_2$, 0.1 mM [γ-^{32}P]ATP and diethylstilbestrol; pH 7.0. The results (means ± SD, N = 3) are given as relative specific activities. The specific activity of the control was 1.20 ± 0.051 nmol PIP/mg protein per min (means ± SD, N = 3).

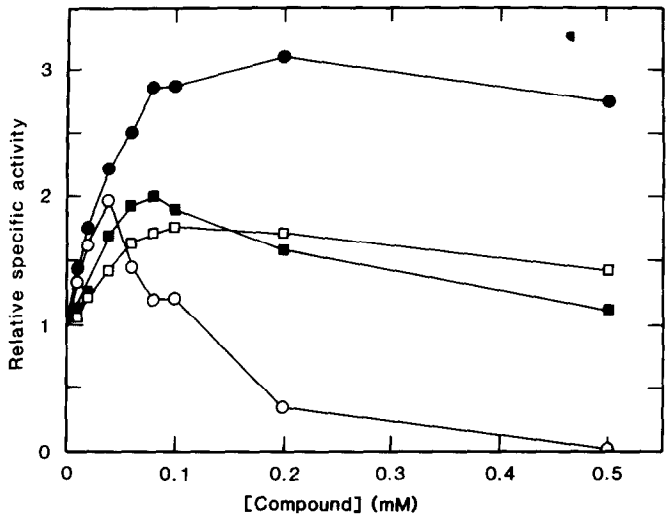

Fig. 3. The effect of different cationic amphiphilic compounds on PI kinase activity. Incubations were performed at 25° for 1 min in a reaction mixture (0.3 ml) containing granule membranes, 30 mM Hepes, 0.1 mM EGTA, 1 mM $MgCl_2$, 0.1 mM [γ-^{32}P]ATP; pH 7.0 and compound 48/80 (●), chlorpromazine (■), desipramine (□) and calmidazolium (○). The results (means, N = 3) are given as relative specific activities. The specific activities of the controls were 1.14 ± 0.007, 1.16 ± 0.040, 1.20 ± 0.050 and 0.90 ± 0.091 nmol PIP/mg protein per min (means ± SD, N = 3) for compound 48/80, chlorpromazine, desipramine and calmidazolium, respectively.

amounts of [^{32}P]PIP, whereas 100% of the label was recovered in the supernatant when 1% (w/v) Triton X-100 was used.

In order to exclude the possibility that inhibition of PI kinase activity by trifluoperazine could be due to a stimulated breakdown of PIP, the effect of trifluoperazine on the level of [^{32}P]PIP in the membrane was investigated. Neither 0.1 mM, nor 0.5 mM trifluoperazine, however, caused any breakdown of [^{32}P]PIP (data not shown).

Effect of other cationic amphiphilic compounds on PI kinase activity

Similar results to those obtained with trifluoperazine were observed for the structurally related cationic amphiphiles desipramine, chlorpromazine,

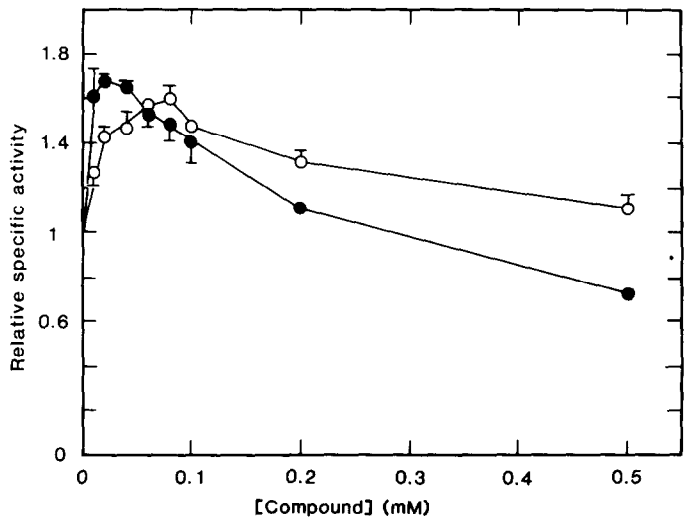

Fig. 4. The effect of neomycin and poly-L-lysine on PI kinase activity. Incubations were performed at 25° for 1 min in a reaction mixture (0.3 ml) containing granule membranes, 30 mM Hepes, 0.1 mM EGTA, 1 mM $MgCl_2$, 0.1 mM [γ-^{32}P]ATP and neomycin (○) or poly-L-lysine (*M*, 3700) (●); pH 7.0. The results (means ± SD, N = 3) are given as relative specific activities. The specific activity of the controls were 1.24 ± 0.106 and 0.89 ± 0.032 nmol PIP/mg protein per min (means ± SD, N = 3) for neomycin and poly-L-lysine (*M*, 3700), respectively.

calmidazolium and compound 48/80 (Fig. 3). Imipramine and propranolol also revealed effects almost identical to desipramine, whereas noradrenaline (0.01–0.5 mM) had no effect on PI kinase activity (data not shown).

Effect of neomycin, polypeptides and polyamines on PI kinase activity

Neomycin sulfate (Fig. 4) and the synthetic polypeptide poly-L-lysine (Figs. 4 and 5A) also revealed a biphasic effect on PI kinase activity. The stimulatory effect revealed the same Mg-dependency as trifluoperazine (Table 1). The inhibition at high concentrations of neomycin and poly-L-lysine could be abolished by increasing the concentration of ATP in the reaction medium (Fig. 5B). A number of basic and amphiphilic polypeptides are known to be calmodulin antagonists [24]. One of these, i.e. mastoparan (Fig. 5A), is a potent stimulator of PI kinase activity in the low micromolar concentration range, whereas both $ACTH_{1-24}$ (Fig. 5A) and $ACTH_{1-39}$ (data not shown) had only a slight inhibitory effect on the PI kinase activity. Spermidine (0.11–1.1 mM) also stimulated the PI kinase activity (148% at 1.1 mM), but no inhibition was observed, whereas putrescine (0.01–0.5 mM) had no effect at all on the enzyme activity (data not shown).

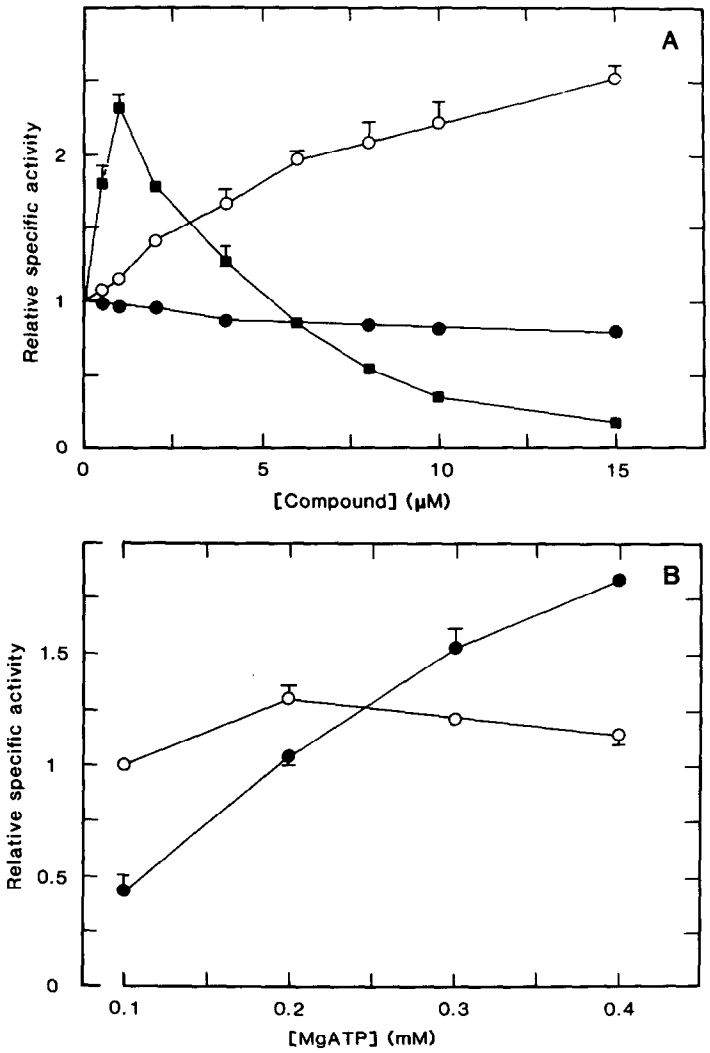

Fig. 5. The effect of polypeptides on PI kinase activity. Incubations were performed at 25° for 1 min in a reaction mixture (0.3 ml) containing granule membranes, 30 mM Hepes, 0.1 mM EGTA, 1 mM $MgCl_2$, [γ-^{32}P]ATP; pH 7.0. (A) The effect of mastoparan (○), $ACTH_{1-24}$ (●) and poly-L-lysine (M_r 41,000) (■) at 0.1 mM [γ-^{32}P]ATP. The results (means ± SD, N = 3) are given as relative specific activities. The specific activity of the controls were 0.92 ± 0.042, 1.27 ± 0.030 and 1.16 ± 0.040 nmol PIP/mg protein per min (means ± SD, N = 3) for mastoparan, $ACTH_{1-24}$ and poly-L-lysine (M_r 41,000), respectively. (B), PI kinase activity in the presence (●) and absence (○) of 10 μM poly-L-lysine (M_r 41,000) at different concentrations of [γ-^{32}P]ATP. The specific activity of the control (0.1 mM ATP) was 1.19 ± 0.092 nmol PIP/mg protein per min (means ± SD, N = 3).

Effect of PIP on PI kinase activity

The possibility of product inhibition of the PI kinase reaction by PIP was investigated in order to explore the mechanism by which cations influence the enzyme activity. PIP was dispersed by sonication in 7.5 mM Hepes, pH 7.0 (as described for PI, see Materials and Methods), and added to the incubation medium at 0.33–66 μg/ml. No effect on PI kinase activity was observed at concentrations of PIP equivalent to those formed in the membrane during a standard incubation (about 1 nmol PIP/mg protein per min). At 66 μg/ml (about 140 nmol/mg protein of granule ghosts) the inhibition of the membrane-bound and solubilized enzyme was 10 ± 4.4 and 23 ± 3.8% (means ± SD, N = 3), respectively.

Effect of EGTA on PI kinase activity

Since PI kinase activity has been shown to be reversibly inhibited by calcium [9], the possibility was considered that phenothiazines could dislocate endogenous bound calcium from the membrane [25], and thereby stimulate the PI kinase activity. The activity was therefore assayed at a constant free Mg^{2+} concentration (see Ref. 9) in the presence of 0.1 mM (control) and 4 mM EGTA, both in a standard granule ghost preparation and in a preparation that previously had been washed three times in a buffer containing 7.5 mM Hepes, 4 mM EGTA, pH 7.0 4 mM EGTA gave a slight increase in activity relative to control, i.e. 26% (N = 6) and 28% (N = 3), respectively.

DISCUSSION

In previous studies we have found that the PI kinase activity of chromaffin granule ghosts is markedly increased by Mg^{2+} (in the mM concentration range) and specifically inhibited by Ca^{2+} (at nM and low μM concentrations) [9], suggesting that this enzyme is a regulated step in the biosynthesis of polyphosphoinositides (PIP and PIP_2). In the present work we have further studied the potential modulation of the PI kinase activity by other hydrophilic as well as by amphiphilic cations, most of which are of considerable pharmacological significance.

Chromaffin granule ghosts are particularly suitable for studies on the membrane-bound form of PI kinase, since they contain neither PIP kinase nor activities that hydrolyze PIP [9]. The cations tested can be divided into two major groups on the basis of their effect on PI kinase activity, i.e. (i) compounds with a biphasic response (stimulation and inhibition), and (ii) those with a selective stimulatory effect. Compounds of the first group, represented by the cationic amphiphile and phenothiazine trifluoperazine, stimulated PI kinase activity in the concentration range of 10–80 μM (Fig. 1) at physiological pH, and in the presence of intracellular concentrations of Mg^{2+} (1 mM $MgCl_2$). At higher concentrations, i.e. 20 mM, no stimulation in addition to that of Mg^{2+} was observed (Table 1), suggesting that trifluoperazine and Mg^{2+} compete for the same binding site in the membrane. Our data stress the importance of using physiological concentrations of Mg^{2+} in the *in vitro* studies of such drugs. In addition, the degree of stimulation of PI kinase activity was not dependent on an intact membrane structure. Thus, the detergent-solubilized enzyme was also stimulated by trifluoperazine in the same concentration range (10–80 μM) as the membrane-bound enzyme (Fig. 1).

The phenothiazine chlorpromazine has been proposed to compete with Ca^{2+} in its binding to phosphoinositides [25]. We have recently shown that PI kinase activity is reversibly inhibited by calcium in a calmodulin-independent manner [9]. The possibility was therefore considered that the stimulatory effect of trifluoperazine was due to a release of membrane-bound calcium. However, the inclusion of 0.2 mM EGTA in the lysis medium during preparation of the granule ghosts and 0.1 mM EGTA in the incubation medium (see Materials and Methods) makes this possibility unlikely. In addition, increasing the concentration of EGTA in the incubation medium to 1 and 4 mM, while keeping the concentration of free Mg^{2+} constant, did not significantly affect PI kinase activity (see Results). The small stimulation observed could be due to small variations in the concentration of free Mg^{2+}. We therefore conclude that the stimulation by trifluoperazine was not due to its properties as calmodulin-antagonist [26] or by releasing membrane-bound calcium [25].

Structurally related cationic amphiphiles (Figs. 3 and 5), as well as the hydrophilic cations neomycin and poly-L-lysine (M_r 3700) (Fig. 4), revealed a stimulatory effect on PI kinase activity in the same concentration range as trifluoperazine. The 41,000 M_r derivative of poly-L-lysine stimulated PI kinase activity at lower concentrations (Fig. 5A). Comparison of the concentrations of the two poly-L-lysine derivatives, giving maximum stimulation of PI kinase activity (20 and 1 μM for the 3700 and 41,000 M_r derivative, respectively), shows that there is a correlation between the number of positive charges and the potency of the stimulatory effect. However, mastoparan (3 lysine residues per molecule) stimulated PI kinase activity more than predicted from the number of positive charges (Fig. 5A), whereas $ACTH_{1-24}$ (4 lysine and 3 arginine residues per molecule) and $ACTH_{1-39}$ both slightly inhibited PI kinase activity (Fig. 5A). These findings indicate that the potency of stimulation of PI kinase activity by amphiphilic peptides is not due simply to their positive charge(s), but is also related to other structural properties.

We conclude on this basis that the stimulation of PI kinase activity by micromolar concentrations of the amphiphilic and hydrophilic cations tested is due to their interaction with a component in the membrane. Binding to the substrate (PI) is unlikely, since one would expect the enzyme activity to be inhibited rather than stimulated, due to reduced availability of the substrate, as has been shown for the phosphatidate phosphohydrolase [27]. However, the possibility cannot be excluded that binding of these drugs to PI can increase the availability of PI to the enzyme [28]. Binding to the product (PIP) could stimulate enzyme activity by decreasing a potential product inhibition. However, we found no inhibition of PI kinase activity by PIP at physiological

concentrations (see Results), making this possibility unlikely. The third and most probable explanation is that the cations bind to a protein component in the membrane, most probably the PI kinase itself. The effectors (e.g. trifluoperazine) seem to bind to the same site as Mg^{2+} in the membrane, but with a higher affinity, since an increase in the concentration of Mg^{2+} from 1 to 20 mM is necessary to remove the effect of 80 μM trifluoperazine (Table 1). This unspecific modulation of PI kinase activity by cations is clearly different from the highly specific inhibition by calcium [9].

An inhibition of PI kinase activity was evident at high micromolar concentrations of all the cationic amphiphilic compounds tested (Figs. 1 and 3, Table 1), as well as the hydrophilic cations neomycin and poly-L-lysine (Figs 4 and 5A). The inhibition by the two latter compounds was due to complexation of ATP, and could be reversed by increasing the ATP concentration (Fig. 5B). The inhibition by the other compounds correlated well with their hydrophobicity as determined by their water:chloroform partition coefficients [29], and a pure inhibitory effect was observed with the non-charged hydrophobic compound diethylstilbestrol (Fig. 2). The inhibition was relatively unaffected by Mg^{2+} (Table 1), and was not due to complexation of ATP (see Results).

Thus, the ability of these effectors to partition into the hydrophobic phase of the membrane seems to determine the inhibitory effect. This intercalation, most potently shown for trifluoperazine, calmidazolium and diethylstilbestrol, did not result in any release of PI kinase from the membrane (see Results), as has been reported for phosphatidate phosphohydrolase [30], or release of phosphoinositides (see Results). Cationic amphiphiles at similar concentrations have been reported to alter the properties of the lipid bilayer, shown as increased permeability of erythrocytes for water and ions [31], as well as inhibited uptake [32] and increased release of catecholamines from chromaffin granules [33].

At least part of the accumulation of polyphosphoinositides seen in the presence of amphiphilic and hydrophilic cations in cells [15, 17, 18] and subcellular fractions [12–14, 16], may be caused by a stimulation of the PI kinase activity. Even if the therapeutic serum concentration of cationic amphiphilic drugs are in the low micromolar concentration range (0.3–1 μM for chlorpromazine [34]), the large distribution volume reflecting binding to membranes [35], indicate that the cellular concentrations are higher, possibly in a concentration range that affects PI kinase activity *in vivo*. Intracellular cations, e.g. spermidine, may also contribute to the regulation of this enzyme *in vivo* [14, 36]. However, noradrenalin, which is found in high concentrations in chromaffin granules *in vivo*, does not seem to modulate PI kinase activity. The stimulation of PI kinase activity by neomycin (Fig. 4) provides an explanation for the observed accumulation of PIP in response to neomycin in the cerebral cortex [12]. Our finding suggests an alternative mode of action of neomycin, i.e. binding to the PI kinase (see above), in addition to its ability to bind polyphosphoinositides and ATP [37]. Finally, our results emphasize that the use of cationic amphiphilic drugs, including the peptide mastoparan, as probes for calmodulin-dependent reactions must be applied with caution.

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