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Muscarinic-agonist and guanine nucleotide activation of polyphosphoinositide phosphodiesterase in isolated islet-cell membranes

Marjorie E. DUNLOP and Richard G. LARKINS
University of Melbourne, Department of Medicine, Royal Melbourne Hospital, Melbourne, Victoria 3050, Australia

Stimulated hydrolysis of the inositol phospholipids phosphatidylinositol 4-phosphate (PtdIns4*P*) and phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5)P_2] was investigated by studying the phosphoinositides produced in a suspended preparation of plasma membranes by transference of ^{32}P from [γ-^{32}P]ATP. At basal Ca^{2+} concentration (calculated free Ca^{2+}, 150 nM) phospholipid hydrolysis was stimulated either by the muscarinic agonists carbamoylcholine and bethanecol or by the addition of the non-hydrolysable analogue of GTP, guanosine 5'-[$\beta\gamma$-imido]triphosphate [p(NH)ppG]. GTP was without effect on basal hyrolysis. Both GTP and p(NH)ppG enhanced the rapid (within 10 s) hydrolysis of PtdIns4*P* and PtdIns(4,5)P_2 induced by carbamoylcholine in a dose-dependent manner. A rightward shift in the competition curve of carbamoylcholine for bound L-[^{3}H]quinuclidinyl benzilate was seen on addition of GTP or p(NH)ppG (100 μM) under phosphorylating conditions. Pretreatment of intact islet cells with *Bordetella pertussis* toxin, islet-activating protein (IAP) or treatment of membranes with IAP under conditions which elicited ADP-ribosylation of a protein of M_r 41000 was without effect on muscarinic binding, phosphoinositide phosphorylation or subsequent hydrolysis by carbamoylcholine. The findings indicate the involvement of a GTP-binding protein in the coupling of the muscarinic receptor to phosphoinositide hydrolysis in the islet cell and suggest that this is distinct from the GTP-binding regulatory component of adenylate cyclase which is covalently modified by IAP.

INTRODUCTION

Activated receptors in many cell types are coupled to guanine nucleotide-binding proteins which regulate either adenylate cyclase (Rodbell *et al.*, 1971; Northup *et al.*, 1980; Hildebrandt *et al.*, 1982; Jakobs *et al.*, 1983) or phosphodiesterase activity (Stryer *et al.*, 1981; Manning & Gilman, 1983; Manganiello *et al.*, 1984). There is considerable evidence to support the participation of such GTP-binding proteins in the coupling of receptors to phosphoinositide phosphodiesterases (phospholipase C) responsible for the hydrolysis of inositol phospholipids (Haslam & Davidson, 1984; Cockcroft & Gomperts, 1985; Litosch *et al.*, 1985; Smith *et al.*, 1985). A number of pharmacological studies have shown the activation of phosphoinositide turnover through activation of the muscarinic cholinergic receptor (Fisher *et al.*, 1983; Brown & Brown, 1984; Jacobson *et al.*, 1985). In the pancreatic islet, insulin release follows muscarinic stimulation (Malaisse *et al.*, 1967; Wollheim *et al.*, 1980), and muscarinic receptors have been demonstrated (Grill & Ostenson, 1983). Carbamoylcholine has been shown to elicit phosphoinositide hydrolysis in intact islets (Best & Malaisse, 1983, 1984) and phosphorylated islet plasma membranes (Dunlop & Malaisse, 1986), and in intact islets to be accompanied by increased inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3], consistent with a phosphodiesteratic activity on phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5)P_2] (Best & Malaisse, 1984). There are many studies which indicate that muscarinic receptors are coupled to the inhibitory guanine nucleotide-binding protein of adenylate cyclase (N_i; M_r 41000). A specific transfer of ADP-ribose from NAD^+ to N_i, promoted by islet-activating protein (IAP), a *Bordetella pertussis* toxin, attenuates the inhibitory effects of muscarinic agonists on adenylate cyclase (Hazeki & Ui, 1981; Kurose *et al.*, 1983). Similarly, evidence has been presented that N_i is the guanine nucleotide-binding protein coupled to phosphoinositide hydrolysis in response to chemotactic peptide (Okajima & Ui, 1984; Nakamura & Ui, 1985; Smith *et al.*, 1985; Volpi *et al.*, 1985; Brandt *et al.*, 1985) and platelet-activating factor (Naccache *et al.*, 1985), but, on the basis of this same experimental criterion (loss of N_i function after IAP-induced ADP-ribosylation), N_i does not appear to be the guanine nucleotide-binding protein involved in the coupling of muscarinic-receptor activation to polyphosphoinositide turnover in chick heart muscle cells (Masters *et al.*, 1985) and 1321N1 human astrocytoma cells (Evans *et al.*, 1985). We have extended an experimental technique using immobilized cell monolayers on positively charged supports to obtain a vesiculated preparation of pancreatic-islet-cell plasma membrane. With this preparation the presence of the kinases for the phosphorylation of phosphatidylinositol, PtdIns4*P* and 1,2-diacylglycerol can be demonstrated, together with the hydrolysis of PtdIns4*P* and PtdIns(4,5)P_2 in response to muscarinic stimulation (Dunlop & Malaisse, 1986). The present study was undertaken to investigate the effect of muscarinic

Abbreviations used: Ins(1,4,5)P_3, inositol 1,4,5-trisphosphate; PtdIns(4,5)P_2, phosphatidylinositol 4,5-bisphosphate; PtdIns4*P*, phosphatidylinositol 4-phosphate; N_i, inhibitory guanine nucleotide-binding protein of adenylase cyclase: IAP, islet-activating protein; p[NH]ppG, guanosine 5'-[$\beta\gamma$-imido]triphosphate; QNB, quinuclidinyl benzilate.

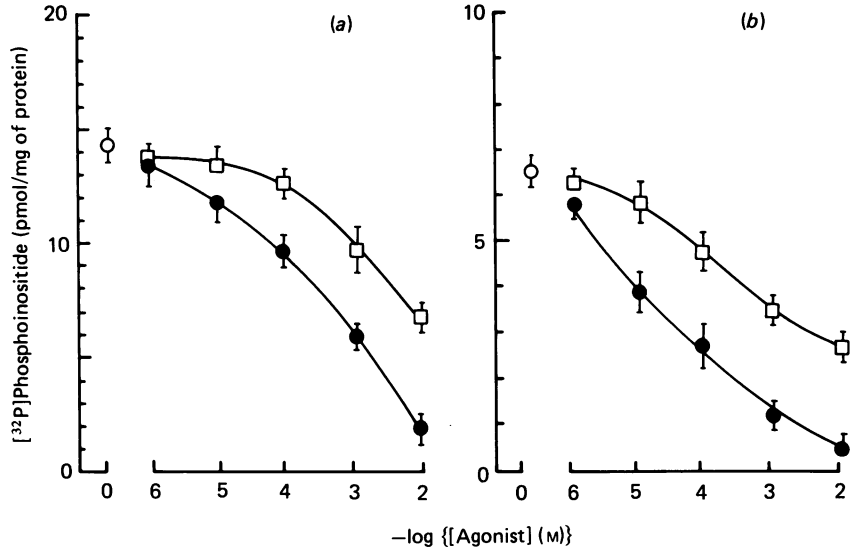

Fig. 1. Effect of muscarinic agonists on polyphosphoinositide content of islet-cell membranes

Islet-cell membranes attached to Cytodex beads and phosphorylated in the presence of [^{32}P]ATP were incubated in the absence (○) or presence of carbamoylcholine (●) or bethanecol (□) at the indicated concentrations for 10 s. ^{32}P content of the phosphoinositides was determined after t.l.c., autoradiography and scintillation counting as described in the Materials and methods section. The results are means ± S.E.M. for four experiments for determination of membrane PtdIns4*P* content (*a*) and PtdIns(4,5)P_2 content (*b*).

agonists and guanine nucleotides on phosphoinositide hydrolysis under conditions favouring membrane phosphorylation and ribosylation, to determine the role of guanine nucleotide regulatory proteins in muscarinic stimulation in the islet cell.

MATERIALS AND METHODS

Preparation of islet-cell membranes

Cells from pancreatic islets of neonatal rats, less than 24 h old, were grown in monolayer culture in medium RPMI 1640 for 3 days and attached to hydrated Cytodex 1 beads as described by Dunlop & Larkins (1984*a*). Cytodex beads with attached membranes were suspended in 50 mM-Tris/HCl buffer, pH 7.7. A membrane fraction was also prepared after washing and homogenization of islet cells in 137 mM-NaCl/2.7 mM-KCl/1.5 mM-KH_2PO_4/8.1 mM-Na_2HPO_4 (pH 7.2) and 150 mM-NaCl/20 mM-Hepes/1 mM-EDTA (pH 7.0) respectively for determination of membrane protein ribosylation. Protein content was determined by the method of Bradford (1976).

Pretreatment of cells with IAP

IAP (final concn. 20 ng/ml) dissolved in 0.1 M-sodium/potassium phosphate buffer containing 2 M-urea, pH 7.0, was added to the cultured cells 0.5–6 h before preparation of membranes.

Incubation of membranes under ribosylating and phosphorylating conditions and labelling of membranes with [^{32}P]NAD$^+$ or ^{32}P transferred from [γ-^{32}P]ATP

Islet-cell membranes attached to Cytodex or prepared by washing and homogenization (protein content 500 μg) were incubated in a ribosylation/phosphorylation buffered mixture containing 1 mM-ATP, 20 mM-thymidine, 20 μM-NAD$^+$, 2.5 mM-$MgCl_2$ and 5 mM-dithiothreitol. In the incubations containing Cytodex-suspended membrane fraction, the ATP (final concn. 1 mM) included [γ-^{32}P]ATP (4 μCi/sample). In incubations containing homogenized membrane preparations, resuspended in20 mM-Hepes/1 mM-EDTA/1 mM-dithiothreitol/10% (w/v) sucrose, NAD$^+$ (final concn. 20 μM) included [α-^{32}P]NAD$^+$ (10 μCi/sample) with or without GTP (100 μM). When present, IAP (final concn. 1.0 μg/ml) was preactivated at 37 °C for 15 min in 10 μl of 40 mM-dithiothreitol/50 mM-Tris/HCl buffer (pH 7.4) containing 5 mM-$MgCl_2$, before the addition of ribosylation/phosphorylation solution (200 μl) and membrane fraction (20 μl). In the absence of added $CaCl_2$ the free Ca^{2+} concentration of this incubation is calculated to be less than 0.1 M, and after the addition of Cytodex-attached membrane fraction less than 0.15 μM (Dunlop & Larkins, 1984*b*).

Separation of proteins labelled with [^{32}P]NAD$^+$

At the end of 15 min incubation in the presence of [α-^{32}P]NAD$^+$, homogenized membrane fractions were centrifuged at 10000 *g* for 1 min and the membrane pellet was washed twice in 10 mM-potassium phosphate buffer containing 150 mM-NaCl and 1 mM-EDTA, pH 7.4.

After solubilization, the membrane proteins (100 μg) were separated by SDS/polyacrylamide-gel electrophoresis (10% separating gel, 2.5% stacking gel) by the method of Laemmli (1970). Gels were stained with Coomassie Blue, destained, dried under vacuum and autoradiographed by using Kodak X-O-mat AR film (90 h at −80 °C).

Hydrolysis of phospholipids in cell membranes labelled with ^{32}P transferred from [γ-^{32}P]ATP

Cytodex beads with attached membranes were incubated as described above for 15 min. Buffered solutions of carbamoylcholine, bethanecol, GTP or p[NH]ppG, to

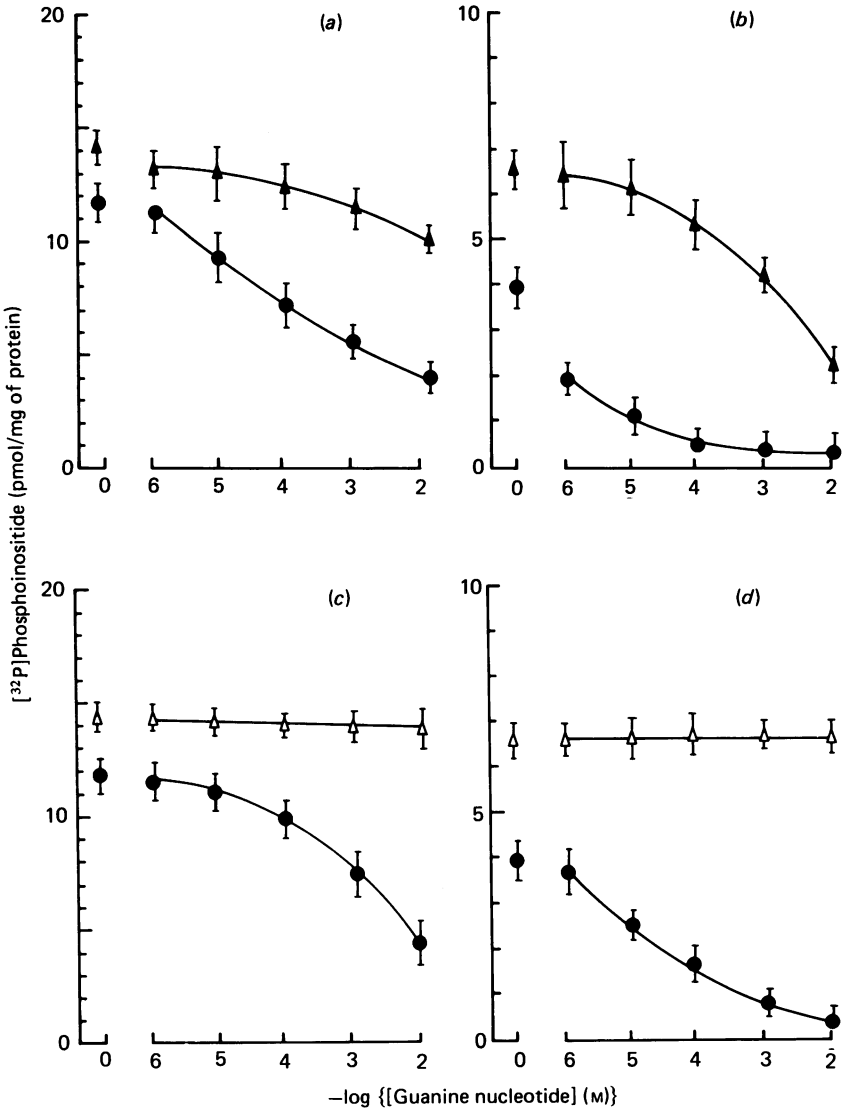

Fig. 2. Effect of guanine nucleotide concentration on polyphosphoinositide content of islet-cell membranes

Phosphorylated islet-cell membranes prepared as described in the legend of Fig. 1 were incubated with p[NH]ppG (*a* and *b*) or GTP (*c* and *d*) at the indicated concentrations for 10 s in the absence (△,▲) or presence (●) of 10 μM-carbamoylcholine. The results are means ± S.E.M. for four experiments for PtdIns4*P* membrane content (*a* and *c*) and PtdIns(4,5)P_2 content (*b* and *d*).

give the final concentrations indicated in the Figure and Table legends, were added swiftly in 20 μl batches. When combined stimuli were present, these were added together. After 10 s the incubation was terminated by addition of chloroform/methanol/13 M-HCl (200:100:1, by vol.) (1.0 ml) and the phospholipids were extracted, separated and quantified after autoradiography as described previously (Dunlop & Malaisse, 1986).

Binding of L-[^{3}H]QNB to islet-cell membranes

Under the incubation conditions described for ^{32}P labelling, membranes were incubated with [^{3}H]QNB (0.3 nM) at 37 °C for 60 min, in the presence of various concentrations of carbamoylcholine. Where indicated, GTP or p[NH]ppG was present at 100 μM. Incubations were terminated by filtration through Whatman GF/C glass microfibre filters and washing with ice-cold homogenization buffer. Non-specific binding was estimated in the presence of atropine sulphate (2.5 μM) and competition binding curves were constructed from the calculated specific binding.

Materials

[γ-^{32}P]ATP (triethylammonium salt) and L-quinuclidinyl [*phenyl*-4(n)-^{3}H]benzilate ([^{3}H]QNB) were obtained from Amersham International; [*adenylate*-^{32}P]NAD$^+$ [di(triethylammonium salt)] was from New England Nuclear; IAP was from Dr. M. Yajima of Kaken Pharmaceuticals, Otsu, Shiga, Japan; GTP and p[NH]ppG were from Boehringer Mannheim; carbamoylcholine chloride and carbamoyl-β-methylcholine chloride (bethanecol) were from Sigma, together with all other chemicals.

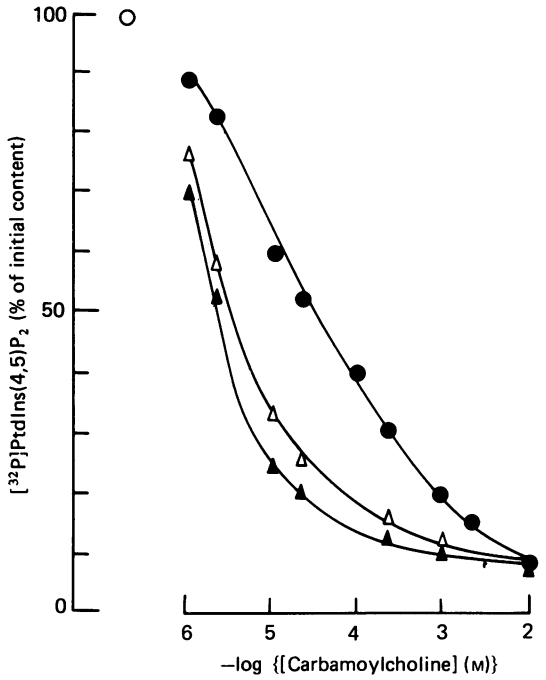

Fig. 3. Effect of carbamoylcholine on PtdIns(4,5)P_2 loss from islet-cell membranes

Phosphorylated islet cell membranes prepared as described in the legend of Fig. 1 were incubated with 0.1 mM-p[NH]ppG (▲) or -GTP (△) together with increasing concentrations of carbamoylcholine, or with carbamoylcholine alone (●), and membrane content of PtdIns(4,5)P_2 was determined. Results are expressed as percentages of the content in the absence of carbamoylcholine. These values were (pmol/mg of protein; mean ± S.E.M. for four determinations): absence of guanine nucleotide, 6.6 ± 0.4; presence of p[NH]ppG, 5.5 ± 0.4; presence of GTP, 6.6 ± 0.5.

RESULTS

Incubation of Cytodex-attached membrane suspension under phosphorylating conditions in the presence of [^{32}P]ATP resulted in the transfer of ^{32}P into PtdIns$_4P$ and PtdIns(4,5)P_2 of 14.4 ± 1.2 and 6.6 ± 0.4 pmol/mg of membrane protein respectively. Incubation of phosphorylated islet membranes with carbamoylcholine caused a loss of PtdIns4P and PtdIns(4,5)P_2 within 10 s. Bethanecol also caused a loss of phosphoinositides, indicating the muscarinic nature of the response, but the effect was not complete in the presence of this agonist over the concentration range studied (Fig. 1). In this membrane preparation, which had not been exposed to Ca^{2+} chelation during preparation, hydrolysis of phosphoinositides was apparent without the addition of Ca^{2+} in the presence of a free Ca^{2+} concentration calculated at < 150 nmol/l.

Hydrolysis induced by p[NH]ppG was essentially similar to that with the muscarinic agonists, as shown in the dose–response curve (Figs. 2*a* and 2*b*). GTP in the absence of a muscarinic agonist was without effect on phosphoinositide breakdown (Figs. 2*c* and 2*d*). The presence of either p[NH]ppG or GTP added simultaneously with the muscarinic agonist enhanced the break-

Table 1. Effect of inclusion of IAP and neomycin sulphate on polyphosphoinositide hydrolysis

Phosphorylated islet cell membranes were incubated with carbamoylcholine (CCh) in the absence or presence of GTP or p[NH]ppG for 10 s, and the ^{32}P contents of PtdIns4P, PtdIns(4,5)P_2 and phosphatidic acid (Ptd) were determined. Where indicated, IAP was present during the phosphorylating period under conditions which support ADP-ribosylation. Neomycin sulphate was added during the 60 s preceding addition of guanine nucleotide. The results are expressed as pmol/mg of protein (means ± S.E.M. for four experiments.).

Addition	Preincubation ...			IAP (1 μg/ml)			Neomycin sulphate (1 mM)		
	None								
	PtdIns4P	PtdIns(4,5)P_2	Ptd	PtdIns4P	PtdIns(4,5)P_2	Ptd	PtdIns4P	PtdIns(4,5)P_2	Ptd
None	14.4 ± 1.2	6.6 ± 0.4	21.9 ± 2.3	14.7 ± 0.9	6.1 ± 0.5	21.7 ± 2.7	14.7 ± 0.9	6.4 ± 0.4	20.9 ± 1.1
GTP (10 μM)	14.0 ± 1.1	6.7 ± 0.5	23.1 ± 2.1	14.2 ± 1.7	6.9 ± 0.6	24.0 ± 1.9	14.5 ± 1.0	6.7 ± 0.5	21.2 ± 1.2
p[NH]ppG (10 μM)	12.9 ± 1.7	5.4 ± 0.6	23.9 ± 1.9	14.4 ± 1.1	6.7 ± 0.4	22.4 ± 1.7	14.2 ± 1.1	6.6 ± 0.5	21.6 ± 2.1
CCh (0.1 mM)	9.9 ± 0.7	2.7 ± 0.3	29.4 ± 2.1	8.7 ± 0.9	2.9 ± 0.3	28.4 ± 2.1	14.7 ± 1.1	6.4 ± 0.6	21.0 ± 1.0
GTP (10 μM); CCh (0.1 mM)	6.2 ± 0.4	0.9 ± 0.3	33.4 ± 2.7	6.4 ± 0.7	1.2 ± 1.1	31.4 ± 2.0			
p[NH]ppG (10 μM); CCh (0.1 mM)	4.1 ± 0.6	0.5 ± 0.3	32.4 ± 2.4	4.4 ± 0.4	1.2 ± 0.3	30.1 ± 2.7			

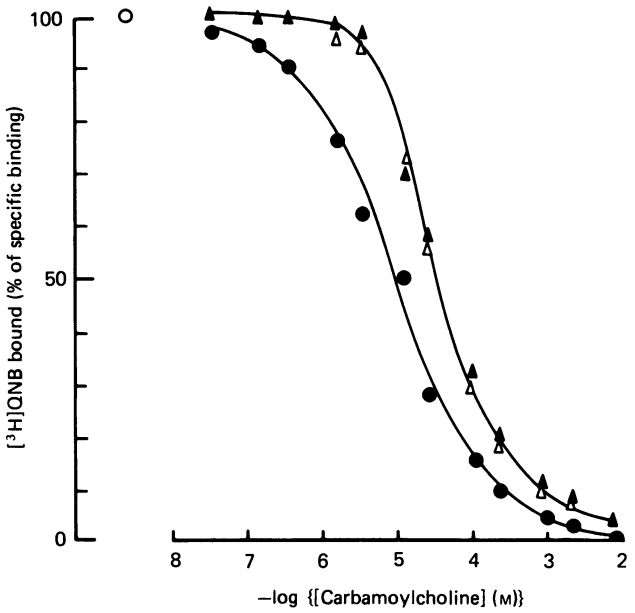

Fig. 4. Effect of guanine nucleotides on muscarinic-receptor binding in islet-cell membranes

Membranes were incubated with [^{3}H]QNB (30 nM) under the conditions described in the Materials and methods section, in the presence of increasing concentrations of carbamoylcholine (●) or carbamoylcholine together with 0.1 mM-p[NH]ppG (▲) or -GTP (△). Results are expressed as percentage of specific binding for an experiment from a single preparation of membranes (triplicate determinations at each point).

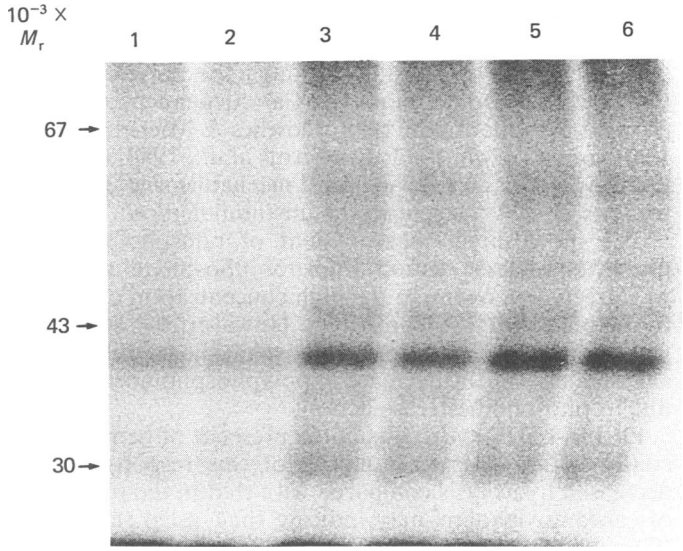

Fig. 5. Autoradiogram of islet membrane proteins after IAP-induced incorporation of [^{32}P]ADP-ribose

Cultured islet cells were exposed to IAP (20 ng/ml) for 0.5 or 6 h and membranes were prepared as described in the Materials and methods section. Membrane proteins (100 μg) were incubated with preactivated IAP (1.0 μg/ml) and [^{32}P]ADP-ribosylated, solubilized and separated by electrophoresis. Lanes 1 and 2, membranes prepared from islets pretreated with IAP for 6 h; lanes 3 and 4, membranes from islets pretreated with IAP for 0.5 h; lanes 5 and 6, control membranes, from islets not pre-exposed to IAP. Ribosylation was carried out in the presence of GTP (0.1 mM) for lanes 2, 4 and 6. M_r marker positions corresponding to 67000 (bovine serum albumin), 43000 (ovalbumin) and 30000 (carbonic anhydrase) are indicated on the left margin.

down of both phosphoinositides in response to muscarinic agonists. Fig. 2 indicates that GTP or p[NH]ppG together with carbamoylcholine (10 μM) produce a dose-dependent fall in content of the membrane phosphoinositides. This effect was seen as a leftward shift in the dose–response curve for carbamoylcholine-induced PtdIns(4,5)P_2 loss (Fig. 3). In the presence of GTP or p[NH]ppG (each 100 μM) the EC_{50} (concn. giving 50% hydrolysis) for carbamoylcholine was estimated to be 25–30 μM, an order of magnitude less than that seen in the presence of carbamoylcholine alone (EC_{50} 300 μM).

Phosphatidic acid formation during the phosphorylation period in these membranes was taken to indicate kinase action on diacylglycerol formed by the action of phosphoinositide phosphodiesterase. The formation of phosphatidic acid (21.9 ± 2.1 pmol of P transferred/mg of protein) was augmented during the stimulated period (Table 1). The effect of carbamoylcholine on phosphoinositide breakdown and subsequent phosphatidic acid synthesis is inhibited by the presence of neomycin sulphate (1 mM) included in the pre-stimulatory period to bind to phosphoinositides.

No effect of inclusion of IAP (1 μg/ml) during the phosphorylation and stimulation period on the hydrolysis of phosphoinositides by carbamoylcholine combined with guanine nucleotides could be demonstrated (Table 1). The presence of muscarinic receptors in this membrane preparation was indicated by the specific binding of the muscarinic antagonist [^{3}H]QNB [90 ± 14 fmol/mg of protein (mean ± S.E.M. for five determinations)]. Under phosphorylating and ribosylating conditions a carbamoylcholine competition curve indicated an IC_{50} (concn. giving 50% inhibition of binding) of 10 μM. In the presence of GTP or p[NH]ppG (each 100 μM), there was an identical rightward shift of the competition curve for carbamoylcholine, giving IC_{50} 300 μM (Fig. 4). Addition of IAP (1 μg/ml) was without effect on [^{3}H]QNB binding and carbamoylcholine displacement curves in the absence or presence of guanine nucleotide (results not shown).

IAP (1 μg/ml) was effective in stimulating the transfer of [^{32}P]NAD^+ to a protein of M_r 41000 in an islet-cell membrane homogenate when it was present during the ribosylation/phosphorylation period (Fig. 5). Moreover, pre-exposure of islet membranes to IAP (20 ng/ml) during culture prevented the subsequent ribosylation by pre-activated IAP (1 μg/ml) (Fig. 5, lanes 1 and 2). The degree of transfer of [^{32}P]NAD^+ was not altered by the presence of GTP under these experimental conditions.

DISCUSSION

The results demonstrate the presence of kinases for the formation of PtdIns4P, PtdIns(4,5)P_2 and phosphatidic acid in membranes prepared from neonatal islet cells, confirming a previous finding in a similar membrane preparation obtained from isolated cells of the adult rat pancreas. Activation of muscarinic receptors in these membranes causes a rapid breakdown of phosphoinositides [PtdIns4P and PtdIns(4,5)P_2] at Ca^{2+} concentrations < 150 nmol/l. This is in accordance with the

view that polyphosphoinositide breakdown induced by muscarinic stimulation occurs before changes in cell Ca^{2+} (Michell *et al.*, 1981), and, although the polyphosphoinositide phosphodiesterase is a Ca^{2+}-dependent enzyme (Akhtar & Abdel Latif, 1978; Downes & Michell, 1981; Van Rooijen *et al.*, 1983; Cockcroft *et al.*, 1984), it may be activated by receptor-coupled mechanisms at concentrations of Ca^{2+} present in the unstimulated cell.

The more direct measurement of phosphoinositide phosphodiesterase action (inositol phosphate release) was precluded, owing to the high concentration of Mg^{2+} in the phosphorylation medium. However, the demonstration of rapid formation of phosphatidic acid is taken as evidence that the decrease in polyphosphoinositides is due to phosphodiesterase action.

PtdIns(4,5)P_2 hydrolysis in the presence of bethanecol confirms the muscarinic nature of this response. The decreased hydrolysis compared with that in the presence of carbamoylcholine may indicate that, as in rat brain (Gonzales & Crews, 1984) and chick heart muscle cells (Brown & Brown, 1984), bethanecol functions as a partial agonist with respect to inositol lipid breakdown. For this reason carbamoylcholine was chosen as the agonist for investigations of guanine nucleotide effects on muscarinic receptor coupling.

Several studies have supported the involvement of guanine nucleotide-binding proteins in receptor-mediated Ca^{2+}-induced processes. Gomperts (1983) showed that the GTP analogue p[NH]ppG introduced into mast cells permeabilized with ATP^{4+} enhanced Ca^{2+}-induced histamine release and, further, Cockcroft & Gomperts (1985) demonstrated that this analogue stimulated hydrolysis of PtdIns4*P* and PtdIns(4,5)P_2 in neutrophil membranes. Similar findings have now been shown for the presence of GTP or its analogues in rat cortical membranes (Gonzales & Crews, 1985) and together with fMet-Leu-Phe in leucocyte membranes (Smith *et al.*, 1985), 5-hydroxytryptamine in salivary gland (Litosch *et al.*, 1985), thrombin in permeabilized human platelets (Knight & Scrutton, 1985), thyrotropin-releasing hormone in GH3 pituitary-cell membranes (Lucas *et al.*, 1985) and vasopressin in rat mammary tumour cells (Guillon *et al.*, 1986).

The inability of GTP alone to cause phosphoinositide hydrolysis is in agreement with the findings in salivary gland (Litosch *et al.*, 1985) and WRK1 cells (Guillon *et al.*, 1986). Although GTP-ase activity in the presence of Mg^{2+} may deplete GTP contents, GTP hydrolysis seems an unlikely explanation for this effect, as GTP and p[NH]ppG are of equivalent potency in affecting [^{3}H]QNB competitive binding and muscarinic-agonist-induced PtdIns(4,5)P_2 hydrolysis. Thus it appears that an agonist is required to induce effective coupling of GTP-binding protein to phospholipase C, whereas p[NH]ppG alone is sufficient for this effect.

The experimental conditions of the present study may be expected to modify muscarinic-receptor binding in a number of ways. The stimulation of phosphoinositide hydrolysis by muscarinic agonists is associated with a low-affinity binding state in rat brain synaptosomal membranes (Fisher *et al.*, 1983) and chick heart muscle (Brown & Brown, 1984). The presence of guanine nucleotides augments this affinity state (Waelbroeck *et al.*, 1982). However, muscarinic-receptor action may be regulated by phosphorylating conditions either through loss of receptor sites or by inhibition of conversion of receptor sites into the low-affinity form (Ehlert *et al.*, 1980; Burgoyne, 1983). Under phosphorylating conditions used in the present study, GTP and p[NH]ppG decrease the apparent affinity of the muscarinic-receptor population, in keeping with the findings in brain (Fisher *et al.*, 1983) and heart (Brown & Brown, 1984).

Using functionally active purified muscarinic receptors and N_i, Haga *et al.* (1985) have shown that brain N_i and the muscarinic receptor are directly coupled to affect GTPase activity of the N_i complex, an effect which may attenuate either adenylate cyclase inhibition or phosphoinositide phosphodiesterase activation under physiological conditions. In contrast with this finding and with studies where N_i is implicated in receptor-coupled phosphoinositide phosphodiesterase (Brandt *et al.*, 1985; Smith *et al.*, 1985; Volpi *et al.*, 1985; Naccache *et al.*, 1985), sufficient evidence is accumulating to propose that a novel guanine nucleotide-regulatory protein may be involved in the coupling of phosphoinositide hydrolysis in certain tissues. In the chick heart and human astrocytoma cells (1321N1) studied by Masters *et al.* (1985), IAP-induced ribosylation of N_i is without effect on inositol phosphate production after muscarinic-receptor activation. The present study is in keeping with those observations. By virtue of lack of effect after IAP exposure, this guanine nucleotide-binding protein(s) also appears to be distinct from the GTP-binding protein (designated N_o) identified in brain (Sternweis & Robinshaw, 1984; Neer *et al.*, 1984). The presence of N_i can be demonstrated in these pancreatic membrane preparations. Although it has been reported that GDP–N_i is the preferred substrate for IAP-induced ADP-ribosylation, as shown previously by Bokoch *et al.* (1983) GTP does not affect ADP-ribosylation of N_i, and significant [^{32}P]NAD^+ transfer to a substrate of M_r corresponding to that of N_i was demonstrated. However, a role for N_i coupled to muscarinic receptors could not be established in these islet cell membranes.

Muscarinic-receptor-mediated inhibition of adenylate cyclase has been shown in cell preparations of many cell types, including endocrine cells (Cronin *et al.*, 1983; Wojcikiewicz *et al.*, 1984), but it is of relevance that in the islet cell preparation we have been unable to demonstrate inhibition of cyclic AMP formation in response to carbamoylcholine (M. E. Dunlop, M. A. Shaw & R. G. Larkins, unpublished work). This is consistent with the stimulatory effect of muscarinic agonists on insulin and glucagon release from β- and α-cells respectively (Iversen, 1973; Ostenson & Grill, 1985) and the positive modulation by increased cyclic AMP concentrations seen in many studies (reviewed by Malaisse & Malaisse-Lagae, 1984) and support the suggestion that muscarinic cholinergic receptors in the pancreatic islet are not coupled to N_i. However, the ability of p[NH]ppG to initiate phosphoinositide loss and the ability of both p[NH]ppG and GTP to sensitize membranes to phosphoinositide loss induced by a muscarinic agonist confirm that a guanine nucleotide-binding protein, distinct from N_i, is involved in polyphosphoinositide phosphodiesterase activation in the islet cell.

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