Biochem. J. (1989) 261, 29–35 (Printed in Great Britain)

Carbachol and histamine stimulation of guanine-nucleotide-dependent phosphoinositide hydrolysis in rat brain cortical membranes

Enrique CLARO,* Agustina GARCIA and Fernando PICATOSTE†
Instituto de Biología Fundamental "V. Villar Palasí" and Departamento de Bioquímica y Biología Molecular, Universidad Autónoma de Barcelona, 08193-Bellaterra, Barcelona, Spain

Guanine nucleotides have been shown to stimulate phosphoinositide breakdown in brain membranes, but no potentiation of such an effect by agonists was demonstrated. We have studied the effect of carbachol and histamine on guanosine 5′-[γ-thio]triphosphate (GTP[S]) stimulation of inositol phosphates formation in [^{3}H]inositol-labelled rat brain cortical membranes. In this preparation, GTP[S] enhancement of phosphoinositide hydrolysis required the presence of MgATP and low Ca^{2+} concentration (100 nM). Carbachol potentiation of the GTP[S] effect was only observed when 1 mM-deoxycholate was also added. Under these conditions, stimulated production of [^{3}H]inositol phosphates was linear for at least 15 min, and [^{3}H]inositol bisphosphate ([^{3}H]IP_2) accounted for approx. 80%, whereas the amount of [^{3}H]inositol trisphosphate ([^{3}H]IP_3) was very low. Stimulation by GTP[S] was concentration-dependent (half-maximal effect at 0.86 μM), and its maximal effect (815% over basal) was increased by 1 mM-carbachol (1.9-fold) and -histamine (1.7-fold). Both agonists decreased the slope index of the GTP[S] concentration/effect curve to values lower than unity, suggesting the appearance of some heterogeneity in the population of guanine-nucleotide-binding proteins (G-proteins) involved. The carbachol and histamine effects were also concentration-dependent, and were inhibited by atropine and mepyramine respectively. Fluoroaluminate stimulated phosphoinositide hydrolysis to a higher extent than GTP[S] plus carbachol, and these stimulations were not additive, indicating that the same polyphosphoinositide phospholipase C-coupled G-protein mediates both effects.

INTRODUCTION

The interaction of a wide variety of hormones and neurotransmitters with their specific membrane receptors results in stimulation of a polyphosphoinositide phospholipase C, with generation of two second messengers, *myo*-inositol 1,4,5-trisphosphate (IP_3), which mobilizes intracellular Ca^{2+}, and 1,2-diacylglycerol, an activator of protein kinase C [1]. As indicated by an increasing amount of evidence, coupling between the agonist-occupied receptors and the phospholipase C appears to be carried out by a G-protein (termed G_p) which behaves like those involved in receptor modulation of adenylate cyclase and retinal cyclic GMP phosphodiesterase [2,3]. Thus, in permeabilized cells and in cell-free preparations from a variety of peripheral tissues, polyphosphoinositide hydrolysis is stimulated by hydrolysis-resistant guanine nucleotides as well as by agonists in a guanine-nucleotide-dependent manner [4–6]. Moreover, AlF_4^-, which is known to activate G-proteins, presumably by causing dissociation of the α and $\beta\gamma$ subunits [7], also stimulates phosphoinositide phospholipase C in membranes from different tissues [8–10], suggesting a structural analogy between G_p and the G-proteins of the adenylate cyclase and transducing systems. In brain tissues, guanine nucleotides and NaF have been shown to stimulate phosphoinositide hydrolysis in [^{3}H]inositol-labelled membranes [11,12] and in membranes incubated with exogenous phosphatidylinositol 4,5-bisphosphate (PIP_2) [13]. However, the guanine-nucleotide-dependent stimulation of the phospholipase C by neurotransmitters was not demonstrated in cell-free preparations from brain, and only in membranes from human astrocytoma 1321N1 cells has the involvement of a G-protein in the agonist stimulation of phosphoinositide hydrolysis been documented [14,15]. In the present work we show the guanine-nucleotide-dependent stimulation of the polyphosphoinositide phospholipase C by muscarinic cholinergic and H_1-histaminergic receptor agonists in a [^{3}H]-inositol-labelled membrane preparation from rat brain cortex.

MATERIALS AND METHODS

Materials

GTP[S], EGTA, NaATP, carbachol, histamine and mepyramine were purchased from Sigma; *myo*-[2-^{3}H]-inositol (14.0–17.1 Ci/mmol) was from New England Nuclear; sodium deoxycholate and atropine were from

Abbreviations used: PI, phosphatidylinositol; PIP, phosphatidylinositol 4-phosphate; PIP_2, phosphatidylinositol 4,5-bisphosphate; IP, inositol monophosphate; IP_2, inositol bisphosphate; IP_3, inositol trisphosphate; IP_4, inositol tetrakisphosphate; GTP[S], guanosine 5′-[γ-thio]triphosphate.

* Present address: Department of Biochemistry, University of Tennessee, Memphis, TN 38163, U.S.A.
† To whom correspondence should be addressed.

Merck; and Dowex 1X8 (100–200 mesh, formate form) was from Bio-Rad.

Preparation of labelled membranes

Male Sprague–Dawley rats were killed by decapitation and the brain was rapidly removed. Cerebral cortices were carefully dissected free of white matter and cross-chopped into 350 μm × 350 μm mini-prisms. Slices were dispersed in Krebs–Henseleit buffer without Ca^{2+} (in mM: 116 NaCl, 4.7 KCl, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 25 Na-HCO_3, 11 glucose), pH 7.4, equilibrated with O_2/CO_2 (19:1) and incubated in the same buffer with 0.3 μM-[³H]inositol for 2 h at 37 °C. At the end of the incubation, the buffer was aspirated and subsequent operations were carried out at 4 °C.

The [³H]inositol-labelled slices were washed three times with 20 mM-Tris/HCl/1 mM-EGTA, pH 7.0, and partially disrupted in the same buffer (approx. 2 ml/g of original tissue) by 50 up-and-down passages through a 1 ml micro-pipette tip. After a 5-fold dilution with the same buffer, the tissue was further homogenized by ten hand strokes in a Teflon/glass homogenizer and centrifuged at 3300 *g* for 10 min. The pellet was re-homogenized in the same volume of buffer by five hand strokes and washed twice by centrifugation and resuspension. The final sediment was resuspended at a protein concentration of 2.5–3.5 mg/ml in the following assay buffer: 10 mM-Tris/maleate, pH 6.8, containing (unless indicated) 3 mM-EGTA, 0.27 mM-$CaCl_2$ (to yield a free Ca^{2+} concentration of 100 nM, calculated as described in ref. [16]), 2 mM-NaATP, 3 mM-$MgCl_2$ and 10 mM-LiCl. Prelabelled membranes were used immediately after preparation.

Phospholipase C assay

Agonist and guanine-nucleotide stimulation of formation of [³H]inositol phosphates in [³H]inositol-labelled rat brain cortical membranes was measured by the method of Berridge *et al.* [17] with some modifications. Incubation mixtures contained portions of membrane suspension (0.2–0.3 mg of protein; 40000–50000 d.p.m.), 1 mM-sodium deoxycholate and, when present, GTP[S] and agonists in a final volume of 250 μl of assay buffer. When used, antagonists were added together with agonists 10 min before GTP[S]. Incubations were carried out for 10 min at 37 °C and were terminated by adding 0.94 ml of chloroform/methanol/10 M-HCl (100:50:1, by vol.). After the tubes had been kept on ice for 15 min, 0.62 ml of methanol/water (1:1, v/v) was added, and the tubes were shaken and centrifuged to separate the phases. Samples (0.75 ml) of the aqueous phases were diluted to 4 ml with water and chromatographed on Dowex 1X8 columns (0.5 ml of resin). Columns were washed with 6 ml of water and 6 ml of 60 mM-sodium formate/5 mM-sodium tetraborate. [³H]Inositol phosphates were routinely eluted together with 8 ml of 1.0 M-ammonium formate/0.1 M-formic acid. In some experiments [³H]inositol phosphates were sequentially eluted with 6 ml each of 0.2 M-ammonium formate/0.1 M-formic acid for [³H]inositol monophosphate ([³H]IP), 0.6 M-ammonium formate/0.1 M-formic acid for [³H]IP_2, and 1.0 M-ammonium formate/0.1 M-formic acid for [³H]IP_3 isomers plus [³H]IP_4, if any. Radioactivity of eluates was measured after adding 15 ml of a toluene/Triton X-100-based scintillation fluid, at approx. 50% efficiency.

Concentration/effect curves for GTP[S] alone or in combination with agonists were fitted to the equation:

$$E = \frac{A^n \cdot E_{max.}}{A^n + EC_{50}{}^n}$$

by using the BMDP AR non-linear-regression iterative program [18] implemented on a VAX 11/785 system. Each point was weighted by the reciprocal of the variance associated with it. The variables E and A were percentage stimulations over basal and GTP[S] concentration respectively. Parameters to be estimated were $E_{max.}$ (maximal effect), n (slope index) and EC_{50} (GTP[S] concentration eliciting half-maximal effect).

RESULTS

Requirements in the composition of the assay buffer

Initial experiments were designed to define the assay requirements for GTP[S] and muscarinic-agonist (carbachol) stimulation of formation of [³H]inositol phosphates in [³H]inositol-prelabelled rat brain cortical membranes. As shown in Fig. 1, when membranes were incubated for 10 min in 10 mM-Tris/maleate, pH 6.8, alone or in presence of 100 nM-Ca^{2+} (3 mM-EGTA, 0.27 mM-$CaCl_2$), no stimulation of phosphoinositide hydrolysis by 10 μM-GTP[S] alone or in combination with 1 mM-carbachol was observed. Basal formation of [³H]inositol phosphates was lower in the EGTA–Ca^{2+}-containing buffer, suggesting that in the absence of chelator the Ca^{2+} concentration may be high enough to stimulate the phospholipase C activity (see below). When 2 mM-NaATP and 3 mM-$MgCl_2$ were included in the Ca^{2+}-buffered medium, GTP[S] stimulated production of [³H]inositol phosphates, but no further activation was

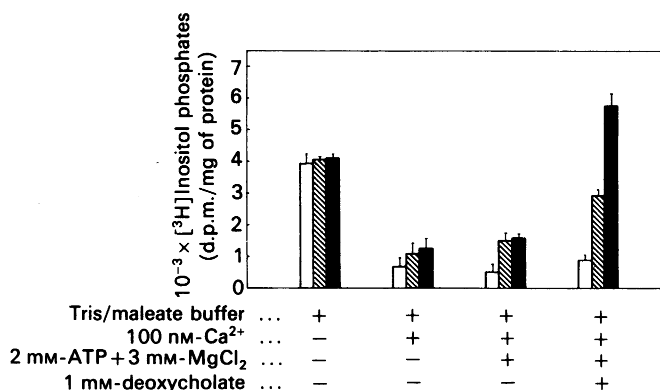

Fig. 1. Assay-buffer requirements for GTP[S] and carbachol stimulation of phosphoinositide hydrolysis in rat brain cortical membranes

Membranes from [³H]inositol-prelabelled rat brain cortical slices (0.2 mg of protein) were incubated for 10 min at 37 °C in 10 mM-Tris/maleate buffer, pH 6.8, in the absence (□) or presence of 10 μM-GTP[S] (▧) or GTP[S] plus 1 mM-carbachol (■) and the additions indicated in the Figure. Total [³H]inositol phosphates produced were measured as described in the Materials and methods section. Concentrations of added EGTA and $CaCl_2$ were calculated to yield a free Ca^{2+} concentration of 100 nM, according to Sasaguri *et al.* [16]. Results are means ± S.E.M. of triplicate determinations.

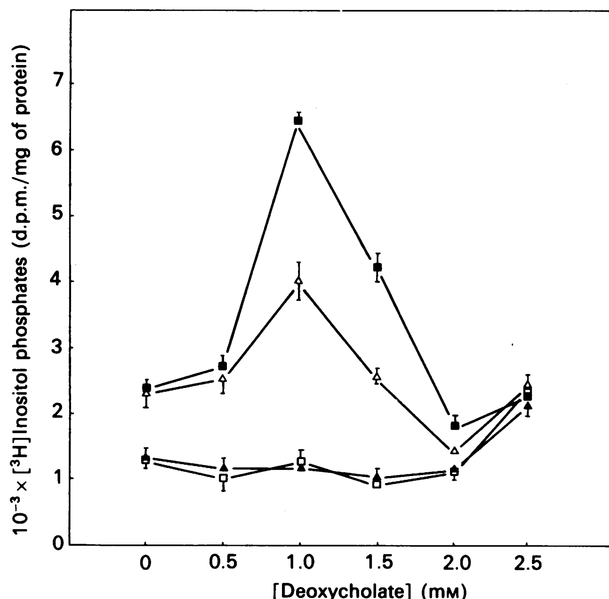

Fig. 2. Effect of deoxycholate on GTP[S] and carbachol stimulation of formation of [^{3}H]inositol phosphates

Prelabelled membranes were incubated in 10 mM-Tris/maleate buffer, pH 6.8, containing 100 nM-Ca^{2+}, 2 mM-ATP, 3 mM-$MgCl_2$ and the indicated concentrations of deoxycholate, in the absence (□) or presence of 1 mM-carbachol (▲), 10 μM-GTP[S] (△) or both (■), for 10 min at 37 °C. Data are means ± S.E.M. of triplicate determinations from a single experiment that was repeated twice with essentially similar results.

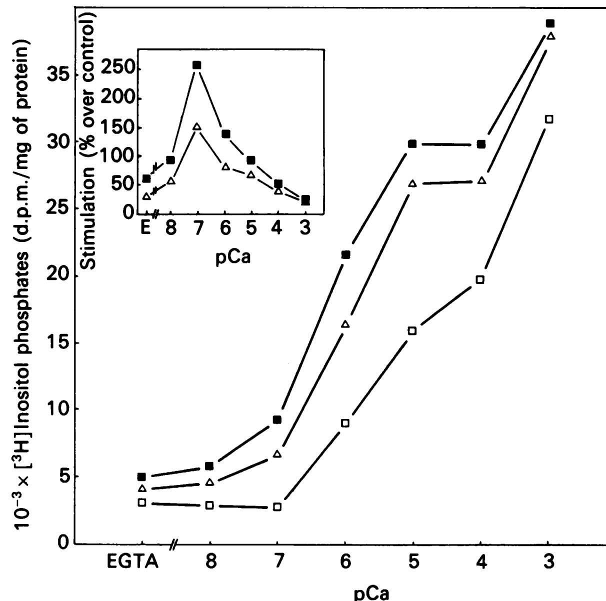

Fig. 3. Effect of Ca^{2+} concentration on basal and GTP[S]- and carbachol-stimulated formation of [^{3}H]inositol phosphates

Prelabelled membranes were incubated in 10 mM-Tris/maleate buffer, pH 6.8, containing 1 mM-deoxycholate, 2 mM-ATP, 3 mM-$MgCl_2$ and increasing concentrations of free Ca^{2+}, established as described in the Materials and methods section. Inset represents stimulation by GTP[S] or GTP[S] plus carbachol expressed as per cent over basal at each Ca^{2+} concentration (E, EGTA). Results are means of triplicate determinations and are representative of data from three similar experiments. Key: □, control; △, 10 μM-GTP[S]; ■, GTP[S] + 1 mM-carbachol.

obtained when carbachol was also present. Under these conditions, the addition of 1 mM-deoxycholate significantly enhanced the response to GTP[S] and allowed the observation of agonist potentiation of the guanine nucleotide effect. Further addition of 10 mM-LiCl to the assay medium did not increase accumulation of [^{3}H]-inositol phosphates (results not shown), suggesting the absence of IP phosphatase in the membrane preparation.

In the absence of GTP[S] no stimulatory effect of carbachol was observed, even when deoxycholate was present. However, the effect of GTP[S] alone or in combination with carbachol was highly dependent on deoxycholate concentration. As illustrated in Fig. 2, maximal GTP[S] and agonist effects were observed at 1 mM-deoxycholate. Higher detergent concentrations resulted in loss of guanine nucleotide and agonist stimulation, and, at concentrations higher than 2.0 mM, deoxycholate alone activated basal phospholipase C activity.

It has been reported that in peripheral tissues Ca^{2+} at micromolar concentrations and higher stimulates phospholipase C activity and that 10–100 nM-Ca^{2+} is required for guanine-nucleotide-induced phosphoinositide hydrolysis [16,19–22]. When we studied the influence of free Ca^{2+} on the GTP[S] and carbachol stimulation of phosphoinositide breakdown in brain cortical membranes, it was also observed that concentrations higher than 100 nM stimulated both basal and GTP[S]- and carbachol-induced formation of [^{3}H]inositol phosphates (Fig. 3). When the stimulation is expressed as per cent over basal (Fig. 3, inset), the maximal phospholipase C modulation capacity of GTP[S] and carbachol was obtained at 100 nM-Ca^{2+}, which is in the concentration range of free cytoplasmic Ca^{2+} in unstimulated cells.

Characteristics of the phospholipase C activation by GTP[S] and agonists

Under standard assay conditions GTP[S] and carbachol stimulated formation of [^{3}H]inositol phosphates linearly with time for at least 15 min. As shown in Fig. 4, the main inositol phosphates formed were [^{3}H]IP_2, which accounted for 70–80 % of the total, and [^{3}H]IP, whereas the amount of [^{3}H]IP_3 accumulated was very low at all times studied.

The effect of GTP[S] alone or in combination with carbachol or histamine was concentration-dependent (Fig. 5). Fitting the concentration/effect curves to a Hill equation as described in the Materials and methods section allowed the estimation of the parameters shown in Table 1. GTP[S] stimulated [^{3}H]inositol phosphates formation with an EC_{50} of 0.86 ± 0.07 μM and a slope index not different from unity, which suggest the involvement of a homogeneous population of G-proteins in the guanine-nucleotide stimulation of the phospholipase C. In the presence of 1 mM-carbachol or -histamine, the maximal GTP[S] effect was increased (188 % and 165 % respectively) and the EC_{50} value slightly decreased. On the other hand, in the presence of agonists the slope index became significantly lower than unity, indicating the appearance of some heterogeneity in the population of G-proteins involved in receptor activation of the phospholipase C.

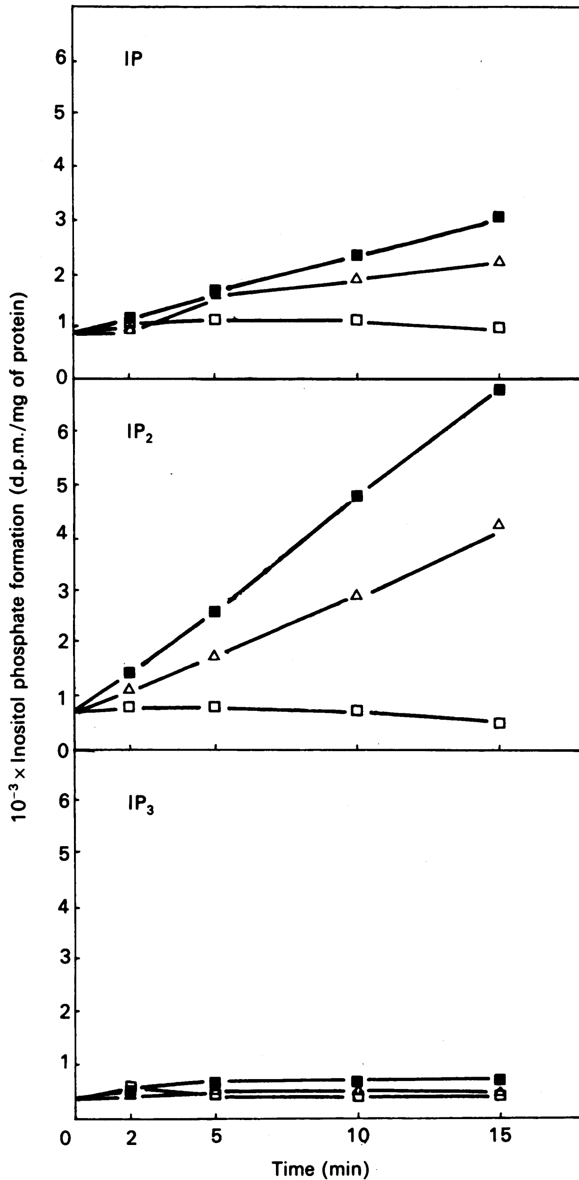

Fig. 4. Time course for GTP[S] and carbachol stimulation of formation of [^{3}H]inositol phosphates

Prelabelled membranes were incubated under standard assay conditions in the absence (□) or presence of 10 μM-GTP[S] (△) or 10 μM-GTP[S] plus 1 mM-carbachol (■) for the indicated times. Individual [^{3}H]inositol phosphates were isolated by anion-exchange chromatography as indicated in the Materials and methods section. Results are means from a single experiment, which was repeated four times with results that differed by less than 10%.

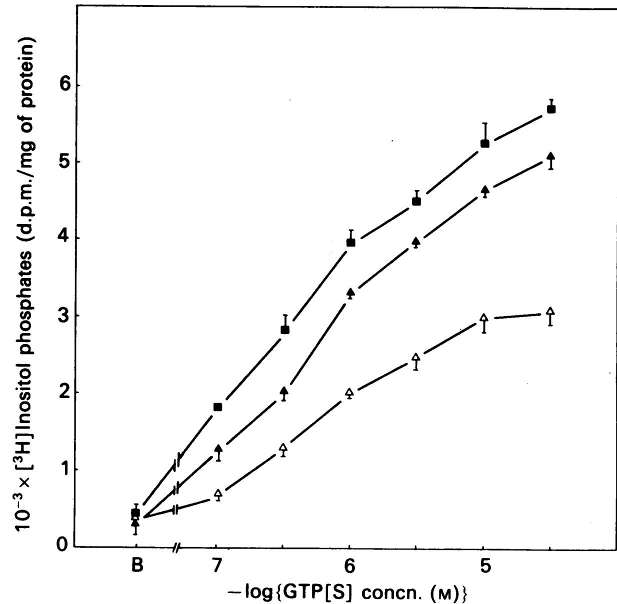

Fig. 5. Concentration/effect curves for the stimulation of formation of [^{3}H]inositol phosphates by GTP[S] alone or in the presence of agonists

Prelabelled membranes were incubated with the indicated concentrations of GTP[S] alone (△) or in combination with 1 mM-carbachol (■) or -histamine (▲). Results are means ± S.E.M. of triplicate determinations from a single experiment which was repeated once with similar results.

Table 1. Concentration/response parameters for the GTP[S] and agonist stimulation of production of [^{3}H]inositol phosphates

The combined data from two independent experiments such as that presented in Fig. 5 were fitted to a Hill equation as described in the Materials and methods section. The parameters estimated (values ± S.E.M.) are the GTP[S] concentration giving half-maximal effect (EC_{50}), the slope factor (n) and the maximal effect as per cent of basal ($E_{max.}$).

	EC_{50} (μM)	n	$E_{max.}$ (% of basal)
GTP[S]	0.86 ± 0.07	0.95 ± 0.04	815 ± 13
GTP[S] + 1 mM-carbachol	0.51 ± 0.06	0.59 ± 0.04	1532 ± 38
GTP[S] + 1 mM-histamine	0.68 ± 0.05	0.73 ± 0.03	1345 ± 15

The effect of carbachol and histamine in the presence of 10 μM-GTP[S] was also concentration-dependent (Fig. 6). The involvement of muscarinic and histamine H_1 receptors was indicated by the abolition of the responses to carbachol and histamine (0.3 mM) by 10 μM concentrations of atropine and mepyramine respectively, whereas atropine showed no effect on the histamine response, nor did mepyramine on that of carbachol, and neither antagonist inhibited the GTP[S] effect (results not shown).

Phospholipase C stimulation by fluoroaluminate

As occurs in the adenylate cyclase system, fluoride ions in the presence of Al^{3+} have been shown to stimulate phosphoinositide phospholipase C in membranes from hepatocytes [8] and other cell types [9,10] apparently by forming the complex AlF_4^-, which interacts with the GDP-bound G-protein and mimics the phosphate group of GTP [7]. Recently, NaF has also been shown to stimulate both endogenous [12] and exogenous [13] PIP_2

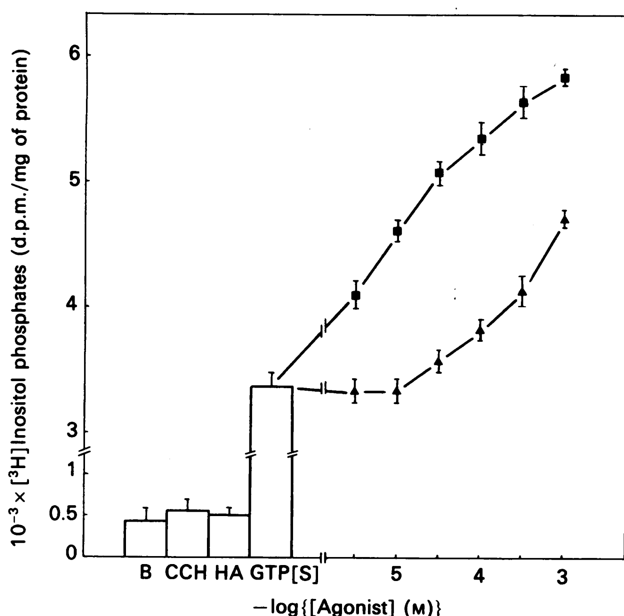

Fig. 6. Concentration/effect curves for carbachol and histamine potentiation of GTP[S]-stimulated formation of [^{3}H]inositol phosphates

Prelabelled membranes were incubated under standard assay conditions in the absence (B) or presence of 1 mM-carbachol (CCH), 1 mM-histamine (HA), 10 μM-GTP[S] or 10 μM-GTP[S] plus increasing concentrations of carbachol (■) or histamine (▲). Agonists were added 10 min before the nucleotide. Results are means ± S.E.M. of triplicate determinations from a representative experiment that was replicated twice.

hydrolysis in brain membranes. We have studied the effect of NaF (5 mM) alone or in combination with $AlCl_3$ (27 μM) on the production of [^{3}H]inositol phosphates in our prelabelled membrane preparation and compared the results with those obtained with 10 μM-GTP[S] and 1 mM-carbachol. In these experiments the buffer was devoid of Ca^{2+}–EGTA, to prevent Al^{3+} sequestration. As expected, NaF + $AlCl_3$ achieved a higher stimulation than NaF (Table 2), and its effect was also higher than that of GTP[S] alone or in combination with carbachol. Moreover, in the presence of NaF + $AlCl_3$ no further stimulation by GTP[S] and carbachol was observed.

DISCUSSION

In the present work, stimulation by GTP[S] of formation of inositol phosphates in a [^{3}H]inositol-labelled membrane preparation from rat brain cortex and muscarinic and H_1-histaminergic enhancement of that effect were found to require incubation of prelabelled membranes in a low-Ca^{2+} medium in the presence of MgATP and deoxycholate. In the absence of detergent only the guanine nucleotide response was observed and, as has been described for membranes of different cell types [8,23–25], the effect required millimolar concentrations of Mg^{2+} and ATP, most probably to allow phosphorylation of PI and PIP and, hence, substrate replenishment. In contrast, Gonzalez & Crews [11,12] were able to observe guanine nucleotide stimulation of phosphoinositide hydrolysis in a rat brain membrane preparation in the absence and presence of Mg^{2+} and ATP and, unexpectedly, in both cases the time course of formation of inositol phosphates showed a similar rapidly declining rate.

Table 2. Fluoride stimulation of formation of [^{3}H]inositol phosphates

Prelabelled membranes were incubated under standard assay conditions (except that Ca^{2+} and EGTA were omitted) in the presence of the indicated compounds. Values are means ± S.E.M. from triplicate determinations made in a single experiment.

Additions	[^{3}H]Inositol phosphates (d.p.m./mg of protein)
None	1946 ± 307
10 μM-GTP[S]	5036 ± 205
10 μM-GTP[S] + 1 mM-carbachol	7030 ± 283
5 mM-NaF	7102 ± 247
27 μM-$AlCl_3$	1717 ± 211
5 mM-NaF + 27 μM-$AlCl_3$	10916 ± 630
5 mM-NaF + 27 μM-$AlCl_3$ + 10 μM-GTP[S]	10410 ± 187*
5 mM-NaF + 27 μM-$AlCl_3$ + 1 mM-carbachol	10422 ± 458*
5 mM-NaF + 27 μM-$AlCl_3$ + 10 μM-GTP[S] + 1 mM-carbachol	11326 ± 392*

* Not significantly different from 5 mM-NaF + 27 μM-$AlCl_3$ ($P < 0.05$, Student's t test).

The presence in the assay medium of sub-critical micellar concentrations of deoxycholate enhanced the effect of GTP[S] and allowed agonist potentiation of the guanine nucleotide response. This effect was not due to direct stimulation by deoxycholate of the phosphoinositide phospholipase C described to occur in brain [26] and other tissues [27,28], since the low detergent concentrations necessary to observe maximal agonist effects did not stimulate basal formation of inositol phosphates. The simplest explanation for the deoxycholate requirement is that partial permeabilization of the membrane vesicles is needed for GTP[S] and agonists to gain access to their respective binding sites, which are presumably located on opposite sides of the membrane. A similar detergent requirement has been reported for membranes from transformed lung epithelial cells [29], but not for membranes from many other cell types, probably reflecting differences in the characteristics of the membrane preparations. Incubation of membranes in the presence of EGTA in a Ca^{2+}-free medium resulted in very low guanine-nucleotide and agonist stimulation of phosphoinositide breakdown. This is in contrast with results from Gonzales & Crews [11], who showed a guanine nucleotide effect in rat brain cortical membranes in the absence of added Ca^{2+}. However, as they pointed out, the actual amount of Ca^{2+} in their preparation was unknown, and in light of our results a small contamination, even with less than micromolar concentrations of the ion, could be responsible for the stimulation they observed. In our preparation, increasing the Ca^{2+} concentration up to 100 nM did not affect basal phosphoinositide hydrolysis, but enhanced GTP[S] and car-

bachol effects, whereas concentrations of 1 μM and higher increased both basal and stimulated formation of inositol phosphates. This is in agreement with studies performed in membrane preparations from several tissues [16,19–22] and further supports the contention that Ca^{2+} concentrations characteristic of a resting cell (approx. 100 nM) allow optimal receptor modulation of the phosphoinositide phospholipase C, and that guanine-nucleotide and agonist activation of the enzyme lowers its Ca^{2+} requirement.

Under our assay conditions the main inositol phosphate formed upon stimulation with GTP[S] alone or in combination with carbachol was $[^3H]IP_2$, whereas the amount of $[^3H]IP_3$ was negligible. These results differ from those reported previously in membranes from several tissues, where IP_3 accumulation is found after several minutes' stimulation [11,14,15,22,25,30]. According to recent data showing that exogenously added PIP_2 is the best phosphoinositide substrate for guanine-nucleotide-stimulated phospholipase C in rat brain cortical membranes [13], $[^3H]IP_3$ is most probably the primary product formed in our membrane preparation, but it can be rapidly dephosphorylated to $[^3H]IP_2$ by the membrane-bound IP_3 phosphatase present in rat brain [31]. Furthermore, the inclusion of detergent in our assay system may have resulted in activation of the enzyme [31]. In prelabelled hepatocyte membranes, Cockcroft & Taylor [8] also found that, upon stimulation with GTP[S], the greatest increase corresponded to IP_2 unless an inhibitor of IP_3 phosphatase was used. Alternatively, $[^3H]IP_2$ could be generated by direct [³H]PIP hydrolysis, as occurs in platelet membranes stimulated with guanine nucleotides and thrombin [23].

The potency of GTP[S] for stimulating phosphoinositide hydrolysis found in this work ($EC_{50} = 0.86$ μM) is in the same order of magnitude as those observed in several peripheral tissues [8,23,32,33], but 30-fold higher than that reported by Gonzales & Crews [11] in rat brain cortical membranes. In our work, the use of a permeabilizing agent could have increased accessibility of GTP[S] to its binding sites, and the inclusion of millimolar concentrations of Mg^{2+} may have facilitated GTP[S] activation of G_p , as has been shown shown to occur for other G-proteins [2].

Carbachol and histamine enhanced maximal response to GTP[S] through interaction with muscarinic and H_1-histaminergic receptors, respectively, and the effect of carbachol was greater than that of histamine, in agreement with data obtained in rat brain cortical slices [34–36]. In addition to the enhancement of the guanine nucleotide maximal effect, agonists slightly lowered the EC_{50} for GTP[S] and decreased the slope factor of its concentration/effect curve to values significantly lower than unity. These effects suggest that agonists may activate a fraction of the phospholipase C-coupled G-proteins by a mechanism that implies moderate changes in GTP[S] potency. Although in binding studies agonists increase G-protein affinity for GTP[³⁵S] with no change in maximal binding capacity [37,38], suggesting that only an increase in GTP[S] potency rather than in maximal response should occur, agonist enhancement of the guanine nucleotide effect with no apparent change in its potency has been consistently found in prelabelled membrane preparations from different cell types [14,15,22,32,39].

In agreement with previous studies [8–10], fluoroaluminate stimulated phosphoinositide breakdown in rat brain cortical membranes, giving further support to the involvement of a G-protein in phospholipase C activation. Indeed, the failure of GTP[S] and carbachol to increase the fluoroaluminate effect suggests that both activating mechanisms are acting on the same pool of G-proteins. Assuming that fluoroaluminate acts by mimicking the γ-phosphate group of GTP in GDP-bound G-proteins [7], and that release of bound GDP is the rate-limiting step in guanine nucleotide activation of G-proteins [2], our results, showing higher efficacy of fluoroaluminate than that found for GTP[S] and carbachol, could be due to the fact that fluoroaluminate stimulation does not require guanine nucleotide exchange. A preliminary account of this work has been published in abstract form [40].

After the present paper had been submitted for review, a study by Chiu *et al.* [41] appeared in which, using a different methodology, they obtained essentially the same results as ours, showing that a G-protein is involved in muscarinic stimulation of phosphoinositide hydrolysis in rat brain cortical membranes.

This work was supported in part by CAICYT Grant PB85-0249. E.C. was a recipient of a Doctoral Fellowship from MEC (Spain).

REFERENCES

1. Berridge, M. J. (1987) Annu. Rev. Biochem. **56**, 159–193
2. Gilman, A. G. (1987) Annu. Rev. Biochem. **56**, 615–649
3. Taylor, C. W. & Merrit, J. E. (1986) Trends Pharmacol. Sci. **7**, 238–242
4. Litosch, I. & Fain, J. N. (1986) Life Sci. **39**, 187–194
5. Cockcroft, S. (1987) Trends Biochem. Sci. **12**, 75–78
6. Fain, J. N., Wallace, M. A. & Wojcikiewicz, R. J. H. (1988) FASEB J. **2**, 2569–2574
7. Bigay, J., Deterre, P., Pfister, C. & Chabre, M. (1985) FEBS Lett. **191**, 181–185
8. Cockcroft, S. & Taylor, J. A. (1987) Biochem. J. **241**, 409–414
9. Sasaki, T. & Hasegawa-Sasaki, H. (1987) FEBS Lett. **218**, 87–92
10. Paris, S. & Pouyssegur, J. (1987) J. Biol. Chem. **262**, 1970–1976
11. Gonzales, R. A. & Crews, F. T. (1985) Biochem. J. **232**, 799–804
12. Gonzales, R. A. & Crews, F. T. (1988) J. Neurochem. **50**, 1522–1528
13. Litosch, I. (1987) Biochem. J. **244**, 35–40
14. Hepler, J. R. & Harden, T. K. (1986) Biochem. J. **239**, 141–146
15. Orellana, S., Solski, P. A. & Brown, J. H. (1987) J. Biol. Chem. **262**, 1638–1643
16. Sasaguri, T., Hirata, M. & Kuriyama, H. (1985) Biochem. J. **231**, 497–503
17. Berridge, M. J., Downes, C. P. & Hanley, M. R. (1982) Biochem. J. **206**, 587–595
18. Dixon, W. F. & Brown, M. W. (1979) Biomedical Computer Programs, P. Series, University of California Press, Berkeley
19. Cockcroft, S. (1986) Biochem. J. **240**, 503–507
20. Martin, T. F. J., Lucas, D. O., Bajjalieh, S. M. & Kowalchyk, J. A. (1986) J. Biol. Chem. **261**, 2918–2927
21. Taylor, S. J. & Exton, J. H. (1987) Biochem. J. **248**, 791–799
22. Rebecchi, M. J. & Rosen, O. M. (1987) Biochem. J. **245**, 49–57

23. Hrbolich, J. K., Culty, M. & Haslam, R. J. (1987) Biochem. J. **243**, 457–465
24. Lucas, D. O., Bajjalieh, S. M., Kowalchyk, J. A. & Martin, T. F. J. (1985) Biochem. Biophys. Res. Commun. **132**, 721–728
25. Straub, R. E. & Gershengorn, M. C. (1986) J. Biol. Chem. **261**, 2712–2717
26. Manning, R. & Sun, G. Y. (1983) J. Neurochem. **41**, 1735–1743
27. Chung, S. M., Proia, A. D., Klintworth, G. K., Watson, S. P. & Lapetina, E. G. (1985) Biochem. Biophys. Res. Commun. **129**, 411–416
28. Bojanic, D., Wallace, M. A., Wojcikiewicz, R. J. H. & Fain, J. N. (1987) Biochem. Biophys. Res. Commun. **147**, 1088–1094
29. Jackowski, S., Rettenmeier, C. W., Sherr, C. J. & Rock, C. O. (1986) J. Biol. Chem. **261**, 4978–4985
30. Exton, J. H. (1988) Hepatology **8**, 152–166
31. Erneux, C., Delvaux, A., Moreau, C. & Dumont, J. E. (1986) Biochem. Biophys. Res. Commun. **134**, 352–358
32. Pfeilschifter, J. & Bauer, C. (1987) Biochem. J. **248**, 209–215
33. Stutchfield, J. & Cockcroft, S. (1988) Biochem. J. **250**, 375–382
34. Brown, E., Kendall, D. A. & Nahorski, S. R. (1984) J. Neurochem. **42**, 1379–1387
35. Gonzales, R. A. & Crews, F. T. (1984) J. Neurosci. **4**, 3120–3127
36. Claro, E., Arbonés, L., García, A. & Picatoste, F. (1986) Eur. J. Pharmacol. **123**, 187–196
37. Smith, C. D., Uhing, R. J. & Snyderman, R. (1987) J. Biol. Chem. **262**, 6121–6127
38. Evans, S. W., Beckner, S. K. & Farrar, W. L. (1987) Nature (London) **325**, 166–168
39. Merrit, J. E., Taylor, C. W., Rubin, R. P. & Putney, J. W. (1986) Biochem. J. **236**, 337–343
40. Claro, E., García, A. & Picatoste, F. (1988) Neurochem. Int. **13**, S160
41. Chiu, A. S., Li, P. P. & Warsh, J. J. (1988) Biochem. J. **256**, 995–999

Received 14 November 1988/3 February 1989; accepted 20 February 1989