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Specific and nonspecific effects of nucleotides on hormone-induced phosphoinositide turnover in permeabilized human pituitary tumour cells (Flow 9000)

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Previous studies have shown that agonist-induced inositol phosphate formation in the human embryonic pituitary cell line Flow 9000 is regulated by guanine nucleotides, and it is likely that a guanine nucleotide-binding protein is involved in coupling receptors to phosphoinositidase C (PIC) [(1986) Biochem.Soc.Trans. 14, 1135–1136]. We have now tested the specificity of various nucleotides in regulating PIC activity in the absence or presence of the hormone cholecystokinin (CCK-8) in saponin-permeabilized [^{3}H]inositol-labelled Flow 9000 cells. We found that all nucleotides tested (i.e. CTP, UTP, ITP, TTP, GTP, GppNHp, GTP[S], ATP, AppNHp and ATP[S]) stimulated total [^{3}H]inositol phosphate ([^{3}H]IP) formation in a dose-dependent manner with similar potency and efficacy. However, only guanine nucleotides significantly enhanced CCK-8 stimulation of [^{3}H]IP production. These results indicate a physiological role for guanine nucleotides in regulating hormone-induced phosphoinositide turnover. In addition, the effects of nucleotides on calcium-dependent PIC activity are discussed.

G-protein; Nucleotide; Phosphoinositide; Ca^{2+}; Cholecystokinin; (Flow 9000 cell)

1. INTRODUCTION

Recent evidence has suggested that receptor-activated phosphoinositidase C (PIC)-mediated phosphoinositide (PI) metabolism, like that of the adenylate cyclase-cAMP pathway, is regulated by guanine nucleotides [1]. It is likely that a guanine nucleotide-binding protein (G-protein) couples Ca^{2+}-mobilizing receptors to PIC. This suggestion is mainly based on the observation that GTP and its nonhydrolysable analogues (GppNHp and GTP[S]) are capable of enhancing agonist-induced PI hydrolysis in either permeabilized cells or membrane preparations [2]. However, more recent studies have questioned the specificity of this guanine nucleotide-dependent effect. Fain and his colleagues [3] found that various nucleotides (e.g. ATP and AppNHp) are equipotent with their guanine nucleotide counterparts in stimulating both cytosolic and plasma membrane PIC activities in a variety of cell types. Oberdisse and Lapetina [4] have shown that GDP-β-S enhances the stimulatory effect of thrombin on inositol phosphate accumulation in permeabilized platelets. In this study, we have investigated the ability of various nucleotides including guanine nucleotides to regulate inositol phosphate formation in saponin-permeabilized Flow 9000 cells.

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Abbreviations: CCK-8, cholecystokinin octapeptide (sulphated); ATP[S], adenosine-5′-*O*-(3-thiotriphosphate); AppNHp, adenylylimidodiphosphate; GTP[S], guanosine-5′-*O*-(3-thiotriphosphate); GppNHp, guanylylimidodiphosphate; GDP-β-S, guanosine-5′-*O*-(2-thiodiphosphate)

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2. MATERIALS AND METHODS

Human embryonic pituitary clonal cells, Flow 9000 (passage: 17–26), were cultured in Ham's F10 medium supplemented with sera and antibiotics at 37°C in a 95% O_2/5% CO_2 atmosphere as described before [5]. Cells were plated at approx. 1.5×10^5 cells/22 mm assay well. After cells were grown to confluence, *myo*-[2-^{3}H]inositol was added at 2 μCi/ml per well for 48 h, at which time isotopic equilibrium would have been achieved [5]. Cells were then permeabilized by saponin treatment (50 μg/ml, 15 min) at room temperature as described before [6]. After thorough washing with buffer, cells were then challenged with various drugs in the presence of 10 mM LiCl in Krebs-Ringer bicarbonate buffer supplemented with glucose in a final volume of 600 μl for 15 min at 37°C. The reaction was terminated by the addition of 3 vols of an ice-cold chloroform/methanol (1:2) solution and water-soluble total [^{3}H]inositol phosphates ([^{3}H]IP, this fraction consists of inositol monophosphates, inositol bisphosphates, inositol trisphosphates and inositol tetrakisphosphate) were separated and quantified by Biorad AG 1 × 8 anion (formate form)-exchange chromatography.

All experiments were performed at least three times. Results, unless otherwise stated, are means ± SE values. EC_{50} values were derived from a dose-response curve using the computer-assisted curve-fitting programme ALLFIT.

Flow 9000 cells were purchased from Flow Laboratories. CCK-8 (sulphated) was obtained from Bachem. All nucleotides were obtained from Boehringer Mannheim. *myo*-[2-^{3}H]Inositol was purchased from NEN/DuPont. All other chemicals were from Fisons.

3. RESULTS AND DISCUSSION

Fig.1 shows that when two concentrations (200 μM and 1 mM) of various nucleotides were tested on the activation of [^{3}H]IP accumulation in saponin-permeabilized Flow 9000 cells, all nucleotide triphosphates were found to be active. In contrast, dibutyryl cAMP (dcAMP), dibutyryl cGMP (dcGMP) and GDP-β-S (200 μM–1 mM) had no effect on [^{3}H]IP formation in these permeabilized cells. The maximal responses (250–340% of controls) were similar for all nucleotide triphosphates tested and were dose-dependent. Previously, we have shown that guanine nucleotides are essential for an agonist-stimulated PI response in saponin-permeabilized Flow 9000 cells [5]. Accordingly, we have tested whether other nucleotide triphosphates will mimic the effect of guanine nucleotides in enhancing CCK-8 stimulation of [^{3}H]IP production. Fig.2 shows a distinct profile of nucleotide-enhanced CCK-8-stimulated [^{3}H]IP elevation in which only GTP or its nonhydrolysable analogues GTP[S] and GppNHp significantly potentiated the CCK-8 effect. The enhancement was highest for GTP[S] and lowest for GTP. This may be due to the fact that GTP is more readily hydrolysed by GTPase than are GTP[S] and GppNHp. This effect of potentiation by guanine nucleotides of hormone-induced [^{3}H]IP formation is suggestive of a role in regulating receptor-mediated PI metabolism under physiological conditions.

Other workers have previously described that millimolar calcium could directly stimulate PIC activity [7,8]. Here we have shown that calcium (10^{-7}–10^{-3} M) stimulated [^{3}H]IP production in permeabilized Flow 9000 cells in a dose-dependent manner (fig.3). GTP[S] (100 μM) significantly shifted the calcium-[^{3}H]IP dose-response curve to the left with EC_{50} values lowered from 17.8 ± 1.4 μM to 1.12 ± 0.08 μM (n = 4). Moreover, GTP[S] increased the maximal stimulation of [^{3}H]IP formation induced by calcium (1 mM). We then went on to test that other nucleotide triphosphates would enhance the calcium-induced PI hydrolysis. Fig.4 shows that all nucleotide triphosphates and their analogues were capable of potentiating calcium-induced [^{3}H]IP formation. In addition, we observed that none of the nucleotides had an effect on [^{3}H]IP production at zero concentration of free calcium (no added calcium) implying that PIC activity is calcium-dependent. Taken together, these results produce two conclusions: (i) only guanine nucleotides significantly enhance hormone-induced inositol phospholipid turnover in permeabilized Flow 9000 cells and (ii) all nucleotide triphosphates and their analogues can directly stimulate [^{3}H]IP formation in a calcium-dependent manner.

Interpretation of these results is complicated by the fact that multiple species of PIC have been

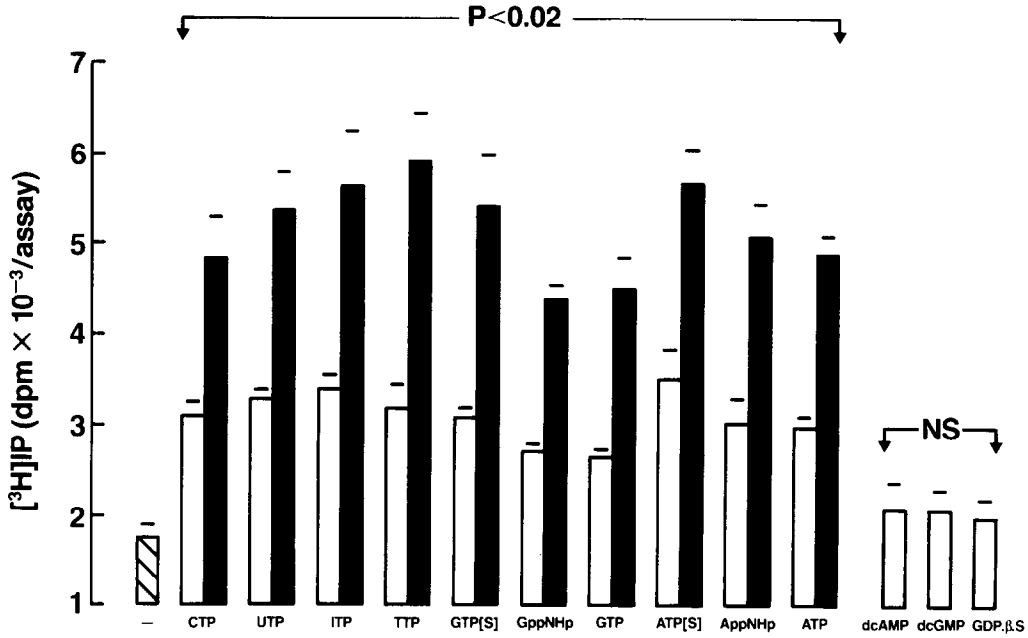

Fig.1. Effects of various nucleotides on [^{3}H]IP formation in saponin-permeabilized Flow 9000 cells. Data presented are means ± SE of 3 separate experiments in triplicate. Nucleotides are added either at 200 μM (open bars) or 1 mM (solid bars). Results are compared to the control value by two tailed unpaired Student's t-test. NS, not significant ($P > 0.05$).

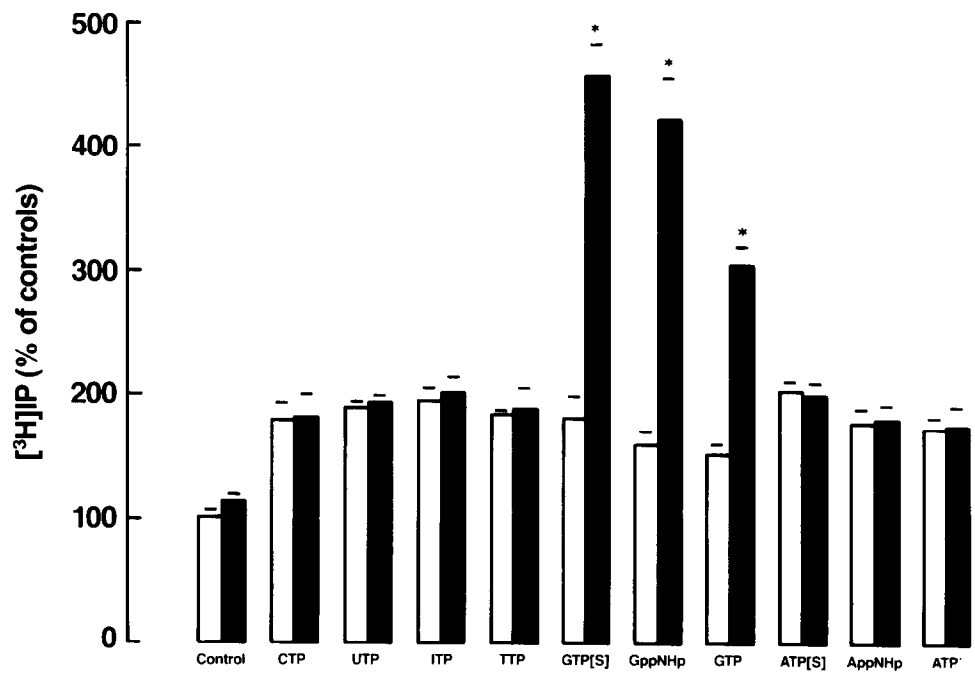

Fig.2. Effects of various nucleotide triphosphates on CCK-8 stimulation of [^{3}H]IP formation. Various nucleotides (200 μM) were added to the cells either in the absence (open bars) or the presence (solid bars) of 10^{-5} M CCK-8. Results presented are means ± SE of 5 independent experiments. * $P < 0.05$ vs corresponding nucleotide stimulation.

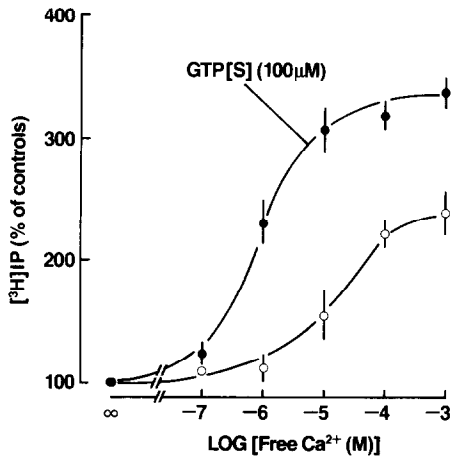

Fig.3. GTP[S] potentiation of calcium-induced [^{3}H]IP accumulation. Data presented are means ± SE of 4 separate experiments in triplicate.

found in various cell types [9,10]. These enzymes tend to have different substrate-specificity in different ionic environments. Moreover, PIC activities have been shown in both cytosolic and plasma membrane fractions [11]. We would like to suggest tentatively that guanine nucleotides may exert their specificity by interacting with the putative G-protein which in turn couples receptors to membrane-bound PIC at the plasma membrane level whilst all nucleotide triphosphates can stimulate cytosolic PIC activity, at least in part, by increasing the affinity of the cytosolic PIC for calcium. Preliminary experiments in our laboratory point to the above possibility in that AlF_4^- stimulation of [^{3}H]IP formation in permeabilized cells was additive with those induced by various nucleotides. However, guanine nucleotide-enhanced CCK-8-induced [^{3}H]IP accumulation was not additive with that stimulated

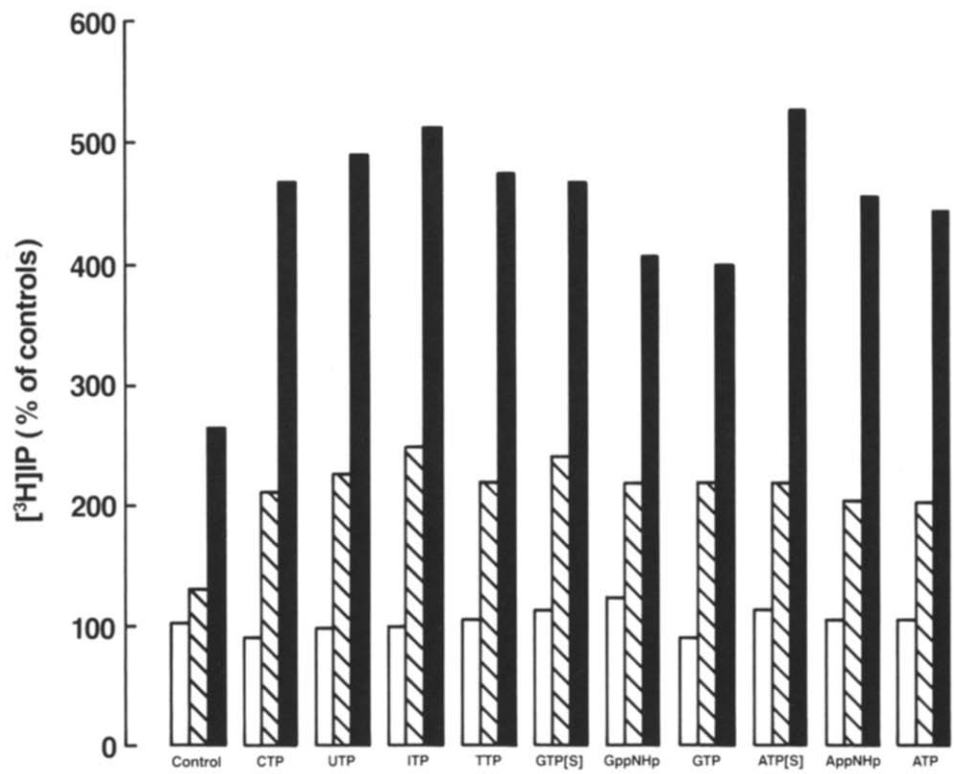

Fig.4. Effects of various nucleotides on calcium-mediated [^{3}H]IP formation. Various nucleotides were incubated with either no added calcium (open bars), 2 μM (hatched bars) or 1 mM (solid bars) calcium. Results shown are means of 4 separate experiments. SE is less than 10% within each experimental point.

by maximal concentrations of AlF_4^-. These preliminary results suggest that the mechanism by which guanine nucleotides enhance the CCK-8 response is similar to that which accounts for AlF_4^- stimulation of PI hydrolysis. The obvious candidate for the limiting factor is the putative G-protein. Additional evidence is needed to confirm this hypothesis.

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