Effects of guanine nucleotides on phosphoinositide turnover in saponin-permeabilized pituitary tumour cells

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Receptor-activated phosphoinositide (PI) metabolism has been implicated in various cellular responses via mobilization of intracellular stored Ca$^{2+}$ and/or activation of protein kinase C (Berridge, 1984; Nishizuka, 1984). This involves a phospholipase C-mediated hydrolysis of a membrane lipid phosphatidylinositol-4,5-bisphosphate (PIP$_2$) to two intracellular second messengers, inositol-1,4,5-trisphosphate (InsP$_3$) and diacylglycerol (DAG). However, it is still not certain how activated receptor is coupled to the catalytic unit of phospholipase C. Recent evidence suggests that a guanine nucleotide-binding protein is involved. For instance, Cockcroft & Gomperts (1985) showed that in permeabilized mast cells, non-hydrolysable analogues of GTP activated inositol phosphate formation as well as histamine release. These analogues also enhance serotonin release and DAG production in permeabilized platelets (Haslam & Davidson, 1984a,b) and stimulate PIP$_2$ breakdown in membranes prepared from different tissues (Gonzales & Crews, 1985; Litosch et al., 1985; UHING et al., 1986). In addition, islet-activating protein, a pertussis toxin which inhibits N$\text{a}^+$ through ADP-ribosylation, inhibits chemotactic peptide-induced arachidonic acid release, PIP$_2$ breakdown and Ca$^{2+}$ mobilization in neutrophils (OKAJIMA & UI, 1984; SMITH et al., 1985; Volpi et al., 1985), while suppressing PIP$_2$ hydrolysis and histamine secretion induced by immunoglobulin E or compound 48/80 in mast cells (NAKAMURA & UI, 1983, 1985). In the present study, we investigated the effects of two non-hydrolysable GTP analogues, guanosine-5'-O-(3-thiotrisphosphate) (GTP$_y$S) and guanylylimidodiphosphate; (InsP$_7$), on PIP$_2$ hydrolysis as measured by the accumulation of inositol phosphate in two pituitary cell lines, Flow 9000 and GH$_1$.

The human embryonic pituitary cell line Flow 9000 and the rat pituitary cell line GH$_1$ were obtained from Flow Laboratories, Herts., U.K. Cells were cultured in serum-supplemented Ham’s F10 medium and were plated at approx. 1.5 x 10$^5$ cells/22-mm well in the 12-well tissue culture cluster (Costar). For experiments investigating the effects of guanine nucleotides on PI turnover, cells were prelabelled with myo-[2,3-$^3$H]inositol (2 $\mu$Ci/well) for 48 h to ensure steady-state labelling. Permeabilization was achieved by incubating the cells in Krebs–bicarbonate buffer containing saponin (50 $\mu$g/ml) at room temperature for 15 min. After treatment with saponin and subsequent washings, 95 ± 5% (n = 4) of the cells were unable to exclude Trypan Blue. This contrasts with control cells incubated in the same buffer but with saponin omitted, where 17 ± 3% (n = 4) of the cells took up Trypan Blue. Permeabilized cells were then exposed to guanine nucleotides and/or other drugs in the presence of 10 mM-LiCl for variable times in a final volume of 0.6 ml. Incubation was terminated by the addition of either three volumes of ice-cold chloroform/methanol (1:2) solution or trichloroacetic acid depending on the method applied to analyse the water-soluble inositol phosphates. $^3$H]inositol phosphates were separated either by Dowex-1 anion-exchange chromatography as previously described (LO et al., 1986) or by the h.p.l.c. method of IRVINE et al. (1985) using a 0.46 cm × 25 cm Partisil SAX 10 high pressure anion-exchange column.

The two non-hydrolysable analogues of GTP, GTP$_y$S and GppNHp, both stimulated $^3$H]inositol phosphate accumulation in permeabilized Flow 9000 cells and GH$_1$ cells in a dose-dependent manner with EC$_{50}$ values of around 5 x 10$^{-3}$ M for GTP$_y$S and 7 x 10$^{-3}$ M for GppNHp in either cell lines. To determine if guanine nucleotides released inositol phosphates from polyphosphoinositides, individual [3-$^3$H]inositol phosphate formation was measured. GTP$_y$S (5 x 10$^{-4}$ M) was found to stimulate the accumulation of all three inositol phosphates, i.e. inositol 1-phosphate (InsP$_1$), inositol 1,4-bisphosphate (InsP$_2$), and InsP$_3$, significantly as early as 5 min in the Flow 9000 cells. The level of stimulation was 180 ± 3%, 228 ± 10% and 225 ± 12% of paired control values for InsP$_1$, InsP$_2$, and InsP$_3$, respectively (n = 3).

In an attempt to elucidate the role of guanine nucleotides on hormone-induced PI breakdown, we investigated the effects of GTP$_y$S on hormone-stimulated $^3$H]inositol phosphate formation in the permeabilized pituitary tumour cells. In the Flow 9000 cells, the C-terminal octapeptide of cholecystokinin (CCK-8) (10$^{-4}$ M) activated a 5-6-fold increase in [$^3$H]InsP$_3$ accumulation, as measured by the accumulation of inositol phosphate in two pituitary cell lines, Flow 9000 and GH$_1$.

Abbreviations used: PI, phosphoinositide; PIP$_2$, phosphatidylinositol 4,5-bisphosphate; InsP$_1$, inositol 1,4,5-trisphosphate; DAG, diacylglycerol; GTP$_y$S, guanosine-5'-O-(3-thiotrisphosphate); GppNHp, guanylylimidodiphosphate; InsP$_2$, inositol 1,4-bisphosphate; CCK-8, C-terminal octapeptide of cholecystokinin; TRH, thyrotropin-releasing hormone.

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Dihydropyridine Ca\(^{2+}\) agonists are potent stimulators of Ca\(^{2+}\) uptake and transmitter release in cultured cerebellar granule cells

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In excitable cells, such as neurons, one of the major mechanisms regulating the level of intracellular Ca\(^{2+}\) is mediated by voltage-sensitive Ca\(^{2+}\) channels (VSCC) (Miller & Freedman, 1984). A variety of compounds, among which 1,4-dihydropyridines (DHP) seem to be the most potent, are able to modulate Ca\(^{2+}\) entry through VSCC (Preuss et al., 1985). It is believed that these substances interact with the VSCC, which, on the basis of binding studies, are abundant in nervous tissues (Murphy & Synder, 1982). However, organic Ca\(^{2+}\) effectors have apparently little functional effect on preparations of nervous tissues (for review see Miller & Freedman, 1984), although under special circumstances such influences have recently been detected (Middlemiss & Spedding, 1985; Nowycky et al., 1985; Turner & Goldin, 1985; Woodward & Leslie, 1986). Here we report that cerebellar granule cells respond, in terms of Ca\(^{2+}\) uptake and transmitter release, to organic Ca\(^{2+}\) effectors at concentrations similar to those influencing functions in non-neuronal excitable cells.

Development of granule cells in culture was associated with the expression of VSCC. Ca\(^{2+}\) uptake, estimated in cells incubated at 37°C for 2.5 min in Hepses-buffered Krebs medium in the presence of 4 pCi of \(^{45}\)CaCl\(_2\)/ml (Zurgil & Zisapel, 1984), was not stimulated by depolarizing extra-

cellular [K\(^+\)] ([K\(^+\)]) at 2 days in vitro (DIV). However, stimulation was detectable by 5 DIV, followed by a sharp increase reaching a plateau by 8 DIV. Experiments were therefore conducted on cultures at 8–12 DIV.

Fig. 1(a) shows that \(^{45}\)Ca\(^{2+}\) uptake by granule cells increased progressively as a function of the concentration of extracellular K\(^+\). The Ca\(^{2+}\) agonist, BAY K 8644 (5 × 10\(^{-5}\) M), had no effect at 5 mM-K\(^+\), but trebled the voltage-sensitive \(^{45}\)Ca\(^{2+}\) uptake at partially depolarizing concentrations of K\(^+\) (at 15 and 25 mM). The stimulation at higher than 50 mM-K\(^+\) was less pronounced, and, at 70 mM, Ca\(^{2+}\) influx was not significantly different in the presence or absence of BAY K 8644. The effect of another Ca\(^{2+}\) agonist of the same class, (+)-(S)-202 791, was similar.

In partially depolarized cells (15 mM-K\(^+\)) the DHP agonists affected \(^{45}\)Ca\(^{2+}\) uptake in a dose-dependent manner, maximal stimulation being attained at 5 × 10\(^{-5}\) M (Fig. 1b). The stimulation declined at concentrations higher than 5 × 10\(^{-5}\) M, and this is consistent with findings in other systems (Schramm et al., 1983). It should be noted that the sensitivity of granule cells to DHP is similar to that of smooth and cardiac muscle preparations (Ho et al., 1985; Rogg et al., 1985).

The component of \(^{45}\)Ca\(^{2+}\) uptake stimulated by 50 mM-BAY K 8644 and 15 mM-KCl was inhibited by the Ca\(^{2+}\) antagonists nifedipine, (−)-(R)-202 791 and D600 but, with the exception of nifedipine, their potency was somewhat lower than in other systems (e.g. Ho et al., 1985; Turner & Goldin, 1985). Furthermore, they were relatively less effective in blocking depolarization-induced Ca\(^{2+}\) uptake in the absence of DHP Ca\(^{2+}\) agonists.

Abbreviations used: VSCC, voltage-sensitive Ca\(^{2+}\) channels; DHP, 1,4-dihydropyridines; [K\(^+\)], extracellular [K\(^+\)]; DIV, days in vitro.