Biochem. J. (1990) 269, 521–526 (Printed in Great Britain)

Stimulation of Ca^{2+}-independent catecholamine secretion from digitonin-permeabilized bovine adrenal chromaffin cells by guanine nucleotide analogues

Relationship to arachidonate release

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The effect of GTP analogues on catecholamine secretion and [^{3}H]arachidonic acid release from digitonin-permeabilized adrenal chromaffin cells was examined. Several GTP analogues stimulated Ca^{2+}-independent exocytosis, with the order of efficacy being XTP > ITP > guanosine 5'-[$\beta\gamma$-imido]triphosphate (p[NH]ppG) > guanosine 5'-[γ-thio]triphosphate (GTP[S]). The stimulatory effect of the GTP analogues appeared to be due to activation of a conventional GTP-binding protein, as it was inhibited by guanosine 5'-[β-thio]diphosphate (GDP[S]). In contrast, Ca^{2+}-dependent exocytosis was only partially inhibited by high doses of GDP[S]. GTP did not stimulate Ca^{2+}-independent exocytosis, but instead was found to inhibit secretion caused by micromolar Ca^{2+}. Arachidonic acid (100 μM) also stimulated Ca^{2+}-independent catecholamine secretion. Determination of the effect of GTP analogues on release of free [^{3}H]arachidonic acid into the medium showed that it was stimulated by GTP[S] but inhibited by GTP, p[NH]ppG, ITP and XTP. The inhibition of [^{3}H]arachidonic acid release by XTP was not prevented by GDP[S]. These results demonstrate that activation of a GTP-binding protein by certain GTP analogues can induce Ca^{2+}-independent secretion in adrenal chromaffin cells and that the effect of GTP analogues on Ca^{2+}-independent secretion can be dissociated from generation of arachidonic acid.

INTRODUCTION

Ca^{2+} acts as a major signal for the initiation of exocytosis in many cell types. In addition, work on permeabilized cells has indicated a role for GTP-binding proteins in exocytosis. In neutrophils [1,2], RINm 5F [3], HL60 [4], parathyroid [5], AtT-20 [6] and adrenal chromaffin [7,8] cells, GTP analogues elicit varying degrees of Ca^{2+}-independent exocytosis. In platelets [9,10] GTP analogues enhance Ca^{2+}-dependent exocytosis, and in mast cells they either enhance Ca^{2+}-dependent exocytosis [11–13] or elicit Ca^{2+}-independent exocytosis [14], depending on the experimental system used. In lactotrophs GTP[S] decreases the magnitude, but increases the rate, of Ca^{2+}-dependent exocytosis [15]. The effects of GTP analogues on Ca^{2+}-independent exocytosis are apparently not due to activation of adenylate cyclase or phospholipase C [3,4,7,8,13,16], and it has been suggested [1,17] that a novel GTP-binding protein, Ge, is closely involved in the exocytotic mechanism. In yeast a small GTP-binding protein (sec 4) is required for exocytotic membrane fusion [18]. It is not known whether the effects of GTP analogues in mammalian cells result from activation of small GTP-binding proteins [19–21] that mediate or regulate membrane fusion or activation of G-proteins that control generation of second messengers.

Receptor activation of phospholipase A_2 and the generation of arachidonic acid and its metabolites occurs through a GTP-binding protein [22] and is a potential site of action of GTP analogues in permeabilized secretory cells [23]. Arachidonic acid has been implicated in membrane fusion processes [24], and recent work on intact HL60 cells [25] has indicated that stimulation of secretion by agonists is correlated with activation of both phospholipase C and phospholipase A_2. Since generation of arachidonic acid or its metabolites has not been excluded as an explanation for the effects of GTP analogues on Ca^{2+}-independent exocytosis, and because of the controversy regarding the extent to which GTP analogues activate Ca^{2+}-independent exocytosis in adrenal chromaffin cells [26,27], we have examined the effects of various GTP analogues on secretion and arachidonate release from digitonin-permeabilized chromaffin cells. We demonstrate that certain GTP analogues can stimulate Ca^{2+}-independent secretion from permeabilized chromaffin cells and that this is not accompanied by an increase in arachidonate release. We conclude that Ca^{2+}-independent secretion from chromaffin cells owing to activation of a GTP-binding protein is not mediated by arachidonic acid or its metabolites.

MATERIALS AND METHODS

Materials

Digitonin was obtained from Calbiochem. GTP[S], p[NH]ppG and GDP[S] were obtained from Boehringer Mannheim, and other nucleotides were from Sigma. [5,6,8,9,11,12,14,15-^{3}H]Arachidonic acid (200 Ci/mmol) was obtained from Amersham.

Isolation and culture of chromaffin cells

Chromaffin cells were isolated from bovine adrenal medullas by enzymic digestion as described by Greenberg & Zinder [28] with modifications [29]. Cells were washed in Ca^{2+}-free Krebs–Ringer buffer, consisting of 145 mM-NaCl, 5 mM-KCl, 1.3 mM-$MgCl_2$, 1.2 mM-NaH_2PO_4, 10 mM-glucose and 20 mM-

Abbreviations used: GTP[S], guanosine 5'-[γ-thio]triphosphate; G-protein, guanine-nucleotide-binding regulatory protein; p[NH]ppG, guanosine 5'-[$\beta\gamma$-imido]triphosphate; GDP[S], guanosine 5'-[β-thio]diphosphate.

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Hepes, pH 7.4 (buffer A), resuspended in culture medium (Dulbecco's modified Eagle's medium with 25 mM-Hepes, 10% foetal-calf serum, 8 μM-fluorodeoxyuridine, 50 μg of gentamycin/ml, 10 μM-cytosine arabinoside, 2.5 μg of fungizone/ml, 25 units of penicillin/ml and 25 μg of streptomycin/ml), plated in 24-well trays at a density of $(0.7–1) \times 10^6$ per well and maintained in culture for 2–7 days.

Permeabilization and assay of catecholamine secretion

Cultures were washed once with buffer A and once with buffer A containing 0.5% fatty-acid-free BSA, and were pre-permeabilized for 6 min by addition of 20 μM-digitonin in 139 mM-potassium glutamate/2 mM-ATP/2 mM-$MgCl_2$/5 mM-EGTA/0.5% fatty-acid-free BSA/20 mM-Pipes, pH 6.5 (0-Ca^{2+} permeabilization buffer). Cultures were then challenged by replacement of buffer with 0-Ca^{2+} permeabilization buffer or permeabilization buffer containing added $CaCl_2$ to give the indicated calculated free Ca^{2+} concentration in the presence or absence of GTP analogues. After 15 min the buffer was removed, centrifuged at 16000 *g* for 2 min, and samples were taken for assay of released endogenous catecholamine by a fluorimetric assay [30]. Total catecholamine remaining within the cells was determined after release of catecholamines with 1% Triton X-100. Catecholamine secretion was calculated as a percentage of total cellular catecholamine. All experiments were carried out at room temperature.

Assay of arachidonic acid release

The assay for release of arachidonic acid was based on that previously described [31]. Cells were incubated with 250 μl (per well) of medium containing 0.5% fatty-acid-free BSA and 10 μCi of [5,6,8,9,11,12,14,15-^{3}H]arachidonic acid/ml for 3 h at 37 °C. The cells were washed, permeabilized and challenged as for secretion experiments. After challenge for 15 min, the buffer on the cells was removed, centrifuged at 16000 *g* for 2 min, and samples were applied to t.l.c. plates with non-radioactive arachidonic acid. The plates were developed with ethyl acetate/acetic acid (100:1, v/v), the arachidonic acid spots were made visible with iodine vapour, and the spots scraped into vials for scintillation counting. Total radioactivity in the cells was determined by solubilizing cells with 1% Triton X-100 and taking samples for scintillation counting.

RESULTS

Effect of GTP analogues on catecholamine secretion

To determine the extent to which GTP analogues activate Ca^{2+}-independent exocytosis in adrenal chromaffin cells, we examined the effects of various GTP analogues that have been shown to be effective in eliciting Ca^{2+}-independent exocytosis in other cell types [4]. In these experiments chromaffin cells were pre-permeabilized with digitonin for 6 min at 0 Ca^{2+} and then challenged with buffers with or without GTP analogues at various free Ca^{2+} concentrations. The effect of GTP analogues on secretion from digitonin-permeabilized chromaffin cells over a range of calculated free Ca^{2+} concentrations is shown in Fig. 1. p[NH]ppG (500 μM), XTP (10 mM) and ITP (10 mM) all elicited Ca^{2+}-independent secretion (i.e. in nominally Ca^{2+}-free buffer) and had little effect on Ca^{2+}-dependent secretion. The magnitude of the increase in Ca^{2+}-independent secretion was always greater with XTP and ITP than with p[NH]ppG. This effect of XTP, ITP and p[NH]ppG on Ca^{2+}-independent secretion was seen in all experiments carried out (11 experiments on 10 separate cell preparations, 6 experiments on 5 separate cell preparations and in 15 experiments on 14 separate cell preparations for XTP,

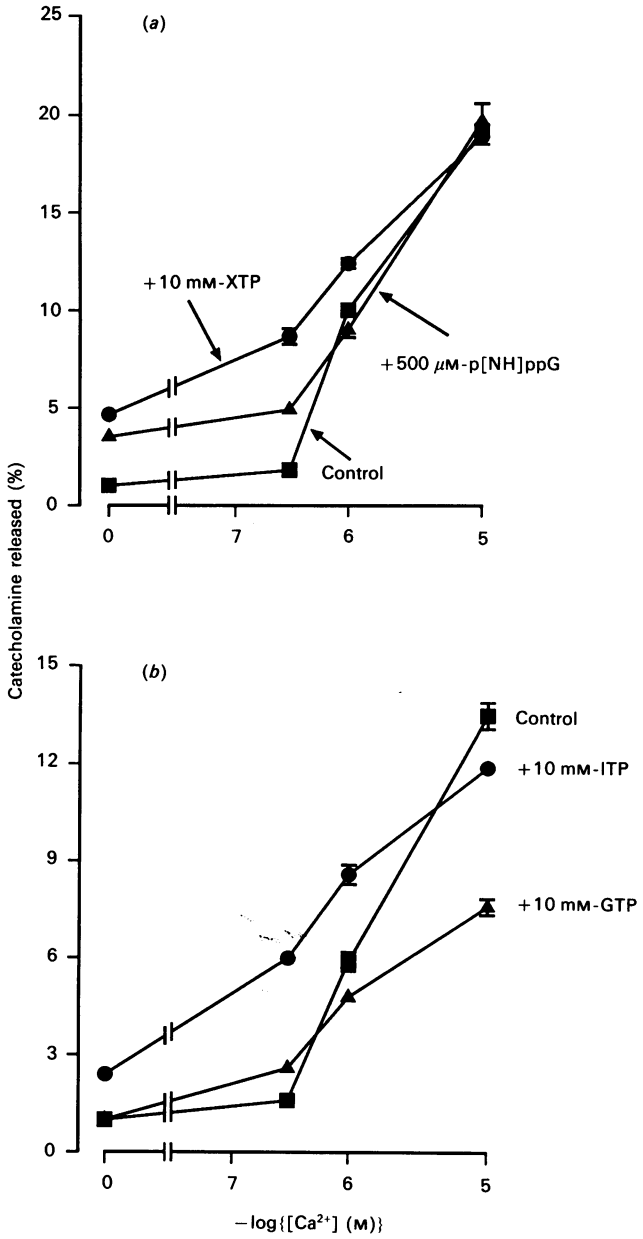

Fig. 1. Effect of GTP analogues on catecholamine secretion from digitonin-permeabilized adrenal chromaffin cells

Chromaffin cells were pre-permeabilized for 6 min in 0 Ca^{2+} and then challenged with buffers containing various concentrations of free Ca^{2+} with no additions or with (*a*) 10 mM-XTP or 500 μM-p[NH]ppG, (*b*) 10 mM-ITP or 10 mM-GTP. The amount of catecholamine released over 15 min was determined, and data are shown as percentages of total cellular catecholamine. The data shown are means ± S.E.M. from four determinations in a typical experiment. In some cases the S.E.M. was smaller than the symbols used.

ITP and p[NH]ppG respectively). The response to p[NH]ppG was maximal at a concentration of 100 μM, whereas that to XTP and ITP was maximal at 10 mM. The effect of GTP[S] (100 μM) on Ca^{2+}-independent secretion was examined in 14 experiments on cells from 11 separate cell preparations, and marginal increases in secretion (see example below) were found in 11 cases, with no effect in the other three (see also [8]). GTP (10 mM) did not elicit Ca^{2+}-independent secretion (Fig. 1*b*), but inhibited secretion caused by micromolar Ca^{2+} (4 experiments).

Effect of preincubation with GTP analogues on catecholamine secretion

In one study of the effect of GTP analogues on secretion from adrenal chromaffin cells, it was reported that when electropermeabilized cells were preincubated with GTP[S] before challenge with micromolar Ca^{2+} GTP[S] resulted in an almost complete inhibition of Ca^{2+}-dependent exocytosis [26]. In the present series of experiments we examined the effect of including GTP[S] (100 μM) or XTP (10 mM) during the 6 min pre-permeabilization period, followed by challenge with or without the analogues. No inhibition by GTP[S] was seen under these conditions. With XTP, inclusion of the analogue during the pre-permeabilization incubation resulted in increased Ca^{2+}-independent secretion, even when the analogue was omitted during the challenge period.

Effect of GDP[S] on secretion caused by GTP analogues and Ca^{2+}

In order to determine whether the effects of GTP analogues were due to activation of a conventional GTP-binding protein, the effect of GDP[S], which would be expected to inhibit GTP-binding-protein activation, was examined. Fig. 2 shows that the stimulatory effect of XTP on Ca^{2+}-independent catecholamine secretion from chromaffin cells was prevented by relatively low doses of GDP[S] (EC_{50} = 25 μM). The effect of a single maximal dose of GDP[S] (750 μM) on Ca^{2+}-independent secretion elicited by p[NH]ppG, XTP, GTP[S] and ITP is shown in Table 1. In this experiment GDP[S] decreased basal secretion and abolished the stimulatory effects of all of the GTP analogues tested. For the purpose of comparison, the effect of GDP[S] on Ca^{2+}-induced secretion was also examined. Doses of GDP[S] that produced substantial inhibition of secretion caused by GTP analogues (Fig. 2) had little effect on Ca^{2+}-induced secretion, which was only partially inhibited even at high doses of GDP[S] (Fig. 3).

Effect of exogenous arachidonic acid on secretion

Arachidonic acid is a possible mediator of the effects of GTP analogues on Ca^{2+}-independent secretion, since its production in several cell types in response to agonists involves coupling to a G-protein [22,23], and GTP[S] activates phospholipase A_2, leading to arachidonic acid release in permeabilized mast cells [32], platelets [33], FRTL5 cells [34] and Swiss 3T3 fibroblasts [35]. Therefore, the effect of exogenous arachidonic acid on secretion from permeabilized chromaffin cells over a range of free Ca^{2+} concentrations was examined (Fig. 4). Arachidonic acid (100 μM) did not affect Ca^{2+}-induced secretion, but elicited a low level of Ca^{2+}-independent secretion (seen in 10 experiments with 8 separate cell preparations).

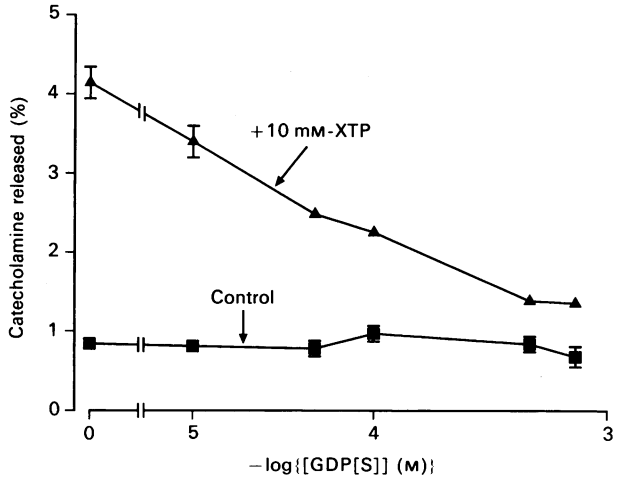

Fig. 2. Inhibition of the stimulatory effects of GTP analogues on Ca^{2+}-independent secretion by GDP[S]

Chromaffin cells were pre-permeabilized for 6 min in 0 Ca^{2+} and then challenged with 0 Ca^{2+} with or without GTP analogues in the absence or presence of GDP[S]. The effect of varying the concentration of GDP[S] on the stimulation of Ca^{2+}-independent secretion by 10 mM-XTP is shown. The amount of catecholamine released over 15 min was determined, and is shown as a percentage of total cellular catecholamine. The data are shown as means ± S.E.M. (n = 6). In some cases the S.E.M. was smaller than the symbols used.

Table 1. Effect of GDP[S] on the stimulatory effect of GTP analogues on Ca^{2+}-independent secretion

Chromaffin cells were pre-permeabilized for 6 min in 0 Ca^{2+} with or without GTP analogues in the absence or presence of 750 μM-GDP[S]. The amount of catecholamine released over 15 min was determined, expressed as a percentage of total cellular catecholamine, and the data are shown as means ± S.E.M. (n = 6).

Stimulus	Catecholamine released (%)		Inhibition by GDP[S] (%)
	No GDP[S]	+GDP[S]	
Expt. 1			
Control	0.73 ± 0.01	0.48 ± 0.01	44
100 μM-p[NH]ppG	1.75 ± 0.02	0.57 ± 0.02	68
10 mM-XTP	2.98 ± 0.1	0.93 ± 0.1	69
Expt. 2			
Control	2.0 ± 0.05	1.4 ± 0.03	30
100 μM-GTP[S]	2.3 ± 0.07	1.3 ± 0.03	44
10 mM-ITP	3.3 ± 0.07	1.5 ± 0.07	55

Effect of GTP analogues on release of [^{3}H]arachidonic acid

Since exogenous arachidonic acid could mimic the stimulatory effect of GTP analogues on Ca^{2+}-independent secretion, the possibility that GTP analogues activated phospholipase A_2 with the subsequent generation of arachidonic acid was considered.

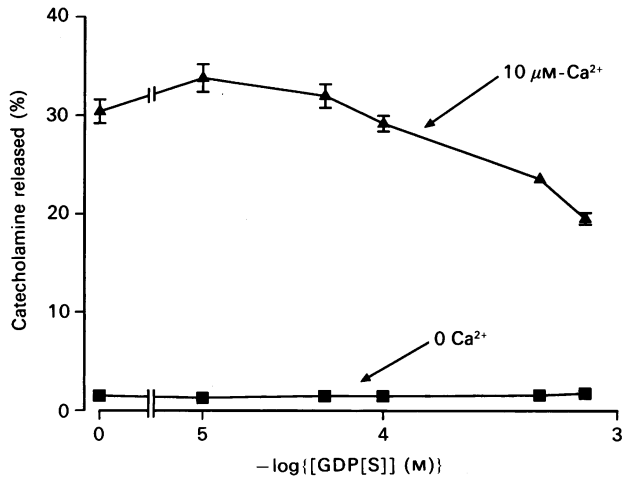

Fig. 3. Effect of GDP[S] on Ca^{2+}-dependent secretion from permeabilized chromaffin cells

After permeabilization, chromaffin cells were challenged with 0 or 10 μM-Ca^{2+} in the presence of various concentrations of GDP[S]. Catecholamine released after 15 min was expressed as a percentage of total cellular catecholamine (n = 4). The data are shown as means ± S.E.M. In some cases the S.E.M. was smaller than the symbols used.

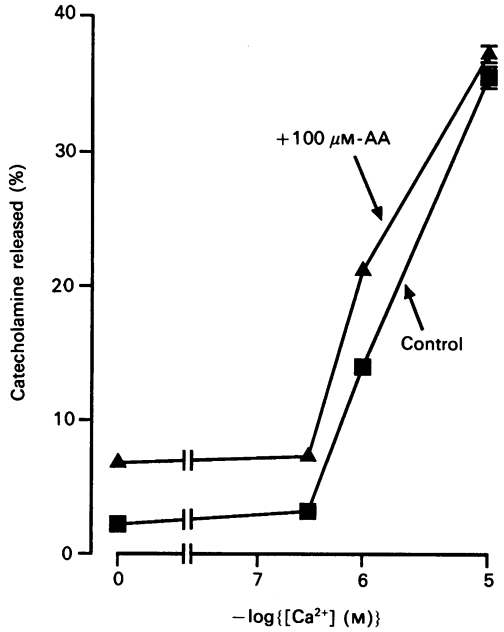

Fig. 4. Effect of arachidonic acid on catecholamine secretion from permeabilized chromaffin cells

After permeabilization, chromaffin cells were challenged with buffers containing various concentration of free Ca^{2+} with or without added 100 μM-arachidonic acid (AA). Catecholamine released after 15 min was expressed as a percentage of total cellular catecholamine and is shown as means ± S.E.M. ($n = 6$).

Intact chromaffin cells were prelabelled with [^{3}H]arachidonic acid, pre-permeabilized with digitonin for 6 min, and the subsequent release of free [^{3}H]arachidonic acid into the medium in the absence or presence of GTP analogues was determined by t.l.c. In control experiments it was confirmed that we could detect increased release of [^{3}H]arachidonic acid from permeabilized chromaffin cells in response to increasing free Ca^{2+} to 10 μM as previously described [30]. In the experiment shown in Fig. 5, catecholamine secretion and [^{3}H]arachidonic acid release in response to GTP analogues were determined in parallel with cells from the same cell preparation. The results for the GTP analogues are ordered to correspond to their efficacy in eliciting Ca^{2+}-independent secretion. Rather than increasing release of [^{3}H]arachidonic acid, the GTP analogues that most effectively elicited Ca^{2+}-independent secretion (XTP, ITP, p[NH]ppG) actually decreased the amount of free [^{3}H]arachidonic acid released into the medium. GTP[S], which had the lowest efficacy for Ca^{2+}-independent secretion, did produce a small increase in [^{3}H]arachidonic acid release. These effects on [^{3}H]arachidonic acid release were seen with two separate cell preparations and, for XTP, with four cell preparations. The decrease in [^{3}H]arachidonic acid release seen with XTP, ITP and p[NH]ppG did not appear to be responsible for the efficacy of these analogues in eliciting Ca^{2+}-independent secretion, since GTP, which does not stimulate secretion, also decreased [^{3}H]arachidonic acid release (Fig. 5). In addition, GDP[S], which abolished the stimulatory effect of XTP on secretion, had no effect on the inhibition of [^{3}H]arachidonic acid release caused by XTP. In a separate experiment [^{3}H]arachidonic acid release was inhibited by 62% in the absence or presence of 750 μM-GDP[S] (Table 2). GDP[S] alone also decreased the release of [^{3}H]arachidonic acid.

DISCUSSION

Stimulatory effects of GTP analogues on exocytosis have been

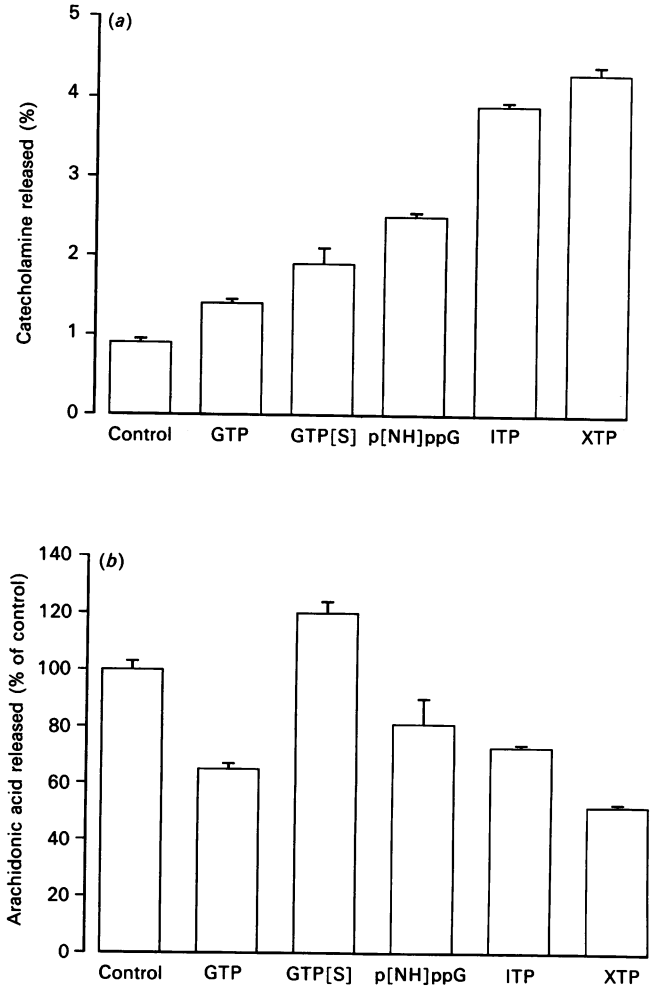

Fig. 5. Effect of GTP analogues on Ca^{2+}-independent catecholamine secretion and release of [^{3}H]arachidonic acid from permeabilized chromaffin cells

In parallel experiments carried out on cells from the same cell preparation, the effect of a range of GTP analogues (*a*) on Ca^{2+}-independent secretion and (*b*) on release of free [^{3}H]arachidonic acid into the medium at 0 Ca^{2+} was determined. The amount of catecholamine or [^{3}H]arachidonic acid released over 15 min was expressed as a percentage (mean ± S.E.M.) of total cellular catecholamine ($n = 6$) or control radioactivity released ($n = 3$) respectively. Control release of [^{3}H]arachidonic acid was 0.51% of total cellular radioactivity. The concentration of GTP analogues was: GTP, 10 mM; GTP[S], 100 μM; p[NH]ppG, 100 μM; ITP, 10 mM; XTP, 10 mM.

Table 2. Effect of GDP[S], XTP and GDP[S] plus XTP on release of [^{3}H]arachidonic acid from permeabilized chromaffin cells

After permeabilization, labelled chromaffin cells were challenged with 0 Ca^{2+} with or without added analogues. The amount of [^{3}H]arachidonic acid released over 15 min was expressed as a percentage of control ($n = 3$). Concentrations of GTP analogues: XTP, 10 mM; GDP[S], 750 μM.

Conditions	[^{3}H]Arachidonic acid released (%)
Control	100 ± 4
GDP[S]	67 ± 3
XTP	38 ± 4
GDP[S] + XTP	38 ± 2

demonstrated in several cell types, and in some cases the analogues stimulate secretion in a Ca^{2+}-independent fashion. The extent to which Ca^{2+}-independent secretion can be stimulated from permeabilized bovine adrenal chromaffin cells by GTP analogues has been controversial. In one study [7], GTP[S] and p[NH]ppG were shown to elicit Ca^{2+}-independent secretion, but other studies have shown that GTP[S] has no or little effect on Ca^{2+}-independent secretion, but enhances Ca^{2+}-dependent secretion [8,27]. In one case, preincubation of electropermeabilized chromaffin cells with GTP[S] was shown to inhibit Ca^{2+}-dependent secretion [26]. The effects of other GTP analogues on permeabilized chromaffin cells have not been examined. We show here that GTP[S] has very little stimulatory effect on Ca^{2+}-independent secretion, but that p[NH]ppG, and to a greater extent XTP and ITP, do stimulate Ca^{2+}-independent secretion that can be blocked by GDP[S], suggesting the involvement of a Ge-like protein that can stimulate Ca^{2+}-independent exocytosis in chromaffin cells. The GTP analogues XTP and ITP were previously shown to stimulate Ca^{2+}-independent secretion from permeabilized HL60 cells [4]. However, the order of efficacy of the GTP analogues in permeabilized chromaffin cells (XTP > ITP > p[NH]ppG > GTP[S]) is almost exactly the reverse of that seen in HL60 cells (GTP[S] > p[NH]ppG > XTP $\simeq$ ITP). Thus it appears that the extent of Ca^{2+}-independent secretion in response to GTP analogues depends on the particular analogue used, and this in turn varies between different cell types. The apparent differing selectivities of Ge for GTP analogues between different cell types may indicate that a family of Ge-like proteins mediates the effects of GTP analogues on Ca^{2+}-independent secretion, or that the results found are complicated in a cell-type-specific manner by the effects of GTP analogues on other GTP-binding proteins. For example, Gp, the activator of phospholipase C, is also selectively activated by different GTP analogues, being most sensitive to GTP[S] [3,4]. It is possible that inhibitory GTP-binding proteins may also be activatable by GTP analogues under some circumstances. In the present study we did not see an inhibitory effect of GTP[S] on Ca^{2+}-dependent secretion as reported previously [26], but a similar inhibitory effect was seen with GTP.

The increased release of catecholamine produced by GTP analogues is unlikely to result from granule lysis rather than exocytosis, since lysis would result in a similar increment in catecholamine release at all Ca^{2+} concentrations. In fact, the effect of GTP analogues appeared to be by the same exocytotic pathway that stimulates release in response to Ca^{2+}, since no additional release was seen with GTP analogues at 10 μM-Ca^{2+}. In addition, the stimulatory effects of GTP analogues on Ca^{2+}-independent secretion from chromaffin cells were prevented by GDP[S], indicating the involvement of a conventional GTP-binding protein rather than some non-specific membrane effect. In contrast, secretion elicited by 10 μM-Ca^{2+} was only partially inhibited, even at high concentration of GDP[S]. This suggests that secretion elicited by micromolar Ca^{2+} may not require Ge or any other GTP-binding protein, but that Ge may be an important regulator of exocytosis at low Ca^{2+} concentrations. Therefore, either elevated Ca^{2+} and Ge may activate exocytosis by separate parallel pathways in chromaffin cells, or the role of Ge may be to sensitize the Ca^{2+}-dependent exocytotic mechanism to low Ca^{2+} concentrations.

Previous studies [8,27] have suggested that the effect of GTP[S] in stimulating Ca^{2+}-dependent secretion in chromaffin cells may be related to the activation of phospholipase C and subsequently protein kinase C. In contrast, the effects of GTP analogues on Ca^{2+}-independent secretion cannot be attributed to activation of this pathway, since stimulation of protein kinase C in chromaffin cells with phorbol esters does not produce Ca^{2+}-independent secretion [7,8,36,37], a result that we confirmed in the present series of experiments (results not shown). Similarly, the effects of GTP analogues is not mediated through Gs, since cyclic AMP [7] or cyclic AMP plus the catalytic subunit of cyclic-AMP-dependent protein kinase did not produce Ca^{2+}-independent secretion in digitonin-permeabilized chromaffin cells (A. Morgan & R. D. Burgoyne, unpublished work). Similar studies have been carried out on other cell types, with the conclusion that GTP analogues may activate secretion in the absence of added Ca^{2+} by a mechanism independent of characterized second-messenger pathways. One possibility remained, however, that GTP analogues stimulated Ca^{2+}-independent secretion through stimulation of phospholipase A_2 and the generation of arachidonic acid and its metabolites. Arachidonic acid was found not to elicit Ca^{2+}-independent secretion from RINm5F cells at doses up to 10 μM [38]. In contrast, we found that in chromaffin cells higher doses of arachidonic acid were able to elicit Ca^{2+}-independent secretion, indicating that release of arachidonic acid might have been the explanation for the effects of GTP analogues. This effect required a high dose of arachidonic acid that may not be of physiological relevance, and our results show that arachidonic acid release is not the explanation for the stimulatory effect of GTP analogues. The GTP analogues that produced the most Ca^{2+}-independent secretion (XTP, ITP and p[NH]ppG) actually inhibited release of free [^{3}H]arachidonic acid from prelabelled cells. Two lines of evidence suggested that the inhibition of arachidonic acid release was also not responsible for the effects of the GTP analogues on Ca^{2+}-independent secretion. First, GTP, which did not stimulate secretion, also decreased the release of arachidonic acid. Second, GDP[S] prevented the stimulation of Ca^{2+}-independent secretion by XTP, but failed to prevent the inhibition of arachidonic acid release by this analogue. In permeabilized platelets, mast cells, FRTL5 cells and Swiss 3T3 fibroblasts [32–35], GTP[S] has been found to stimulate arachidonic acid release; no other GTP analogues were examined. Consistent with these results was our finding that GTP[S] stimulated a small increase in arachidonic acid release in permeabilized chromaffin cells.

In conclusion, our results show that certain GTP analogues can stimulate Ca^{2+}-independent secretion from permeabilized adrenal chromaffin cells, and that this effect can be dissociated from arachidonic acid release. This suggests that exocytosis in chromaffin cells could be stimulated at low Ca^{2+} levels through activation of a novel GTP-binding protein, Ge [1,17], which either activates a still unknown second-messenger pathway or directly participates in the exocytotic machinery.

This work was supported by a project grant from the M.R.C.

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Received 29 January 1990/6 April 1990; accepted 18 April 1990