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Activation of exocytosis by GTP analogues in adrenal chromaffin cells revealed by patch-clamp capacitance measurement

Robert D. Burgoyne*, Susan E. Handel

The Physiological Laboratory, PO Box 147, Liverpool L69 3BX, UK

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Abstract

The role of GTP-binding proteins in exocytosis in bovine adrenal chromaffin cells was examined using patch-clamp capacitance measurement. Internal dialysis with the non-hydrolysable GTP analogue guanosine 5′-[$\beta\gamma$-imido]triphosphate and xanthosine triphosphate (XTP) activated a capacitance increase. Exocytosis triggered by XTP was blocked by guanosine 5′-[β-thio]diphosphate (GDPβS) but Ca^{2+}-induced exocytosis was unaffected. The capacitance increase due to XTP could not be explained by Ca^{2+} mobilisation since Ins(1,4,5)P_3 and caffeine did not mimic the response. Chromaffin cells appear to possess a Ca^{2+}-independent pathway for exocytosis that involves GTP-binding proteins. The magnitude of the response to XTP suggested that GTP analogues stimulate both exocytosis and recruitment of secretory granules.

Key words: Exocytosis; Chromaffin cell; GTP-binding protein; Secretion; Adrenal; Calcium

1. Introduction

Calcium is the major signal for the triggering of regulated exocytosis in many cell types [1]. In others, particularly myeloid cells, a full exocytotic response can be activated, following cell permeabilisation, by non-hydrolysable GTP analogues [2] or requires both Ca^{2+} and a GTP analogue [3]. This has lead to the idea that the exocytotic machinery involves a GTP-binding protein, termed G_E [4,5]. In mast cells the critical GTP-binding protein may be a heterotrimeric G-protein [6]. The ability of GTP-analogues to stimulate release has been examined in a variety of cell types using permeabilised cells [1,4,5] and the ability of the analogues to activate exocytosis has often been confirmed by patch-clamp capacitance as a direct measure of secretory vesicle incorporation into the plasma membrane [7–11].

In adrenal chromaffin cells, Ca^{2+} appears to be the signal for exocytosis. Nevertheless, GTP analogues do stimulate catecholamine release from permeabilised cells, though to a lesser extent than Ca^{2+}, under conditions where Ca^{2+} has been clamped to very low levels [12–14]. Non-hydrolysable analogues of GTP and other analogues such as xanthosine triphosphate (XTP) stimulate catecholamine release from digitonin-permeabilised chromaffin cells [13]. Since catecholamine release could occur from permeabilised cells as consequence of intracellular granule lysis as well as exocytosis it is important to establish that GTP analogues do activate exocytosis in chromaffin cells. Patch-clamp capacitance measurement has been used to study Ca^{2+}-dependent exocytosis in chromaffin cells [15–17] but the effects of GTP analogues have not been assessed using this technique. We demonstrate here that GTP analogues activate an increase in membrane capacitance, and thus exocytosis, consistent with a role for a GTP-binding protein in a pathway leading to exocytosis in chromaffin cells. The magnitude of the capacitance increase suggests that GTP analogues can act not only at a late step in exocytosis via the putative G_E but also in the activation of secretory granule recruitment.

2. Materials and methods

Bovine adrenal chromaffin cells were dissociated from the adrenal medulla and maintained in culture in 35 mm diameter plastic Petri dishes at a density of 10^6 cells per dish as described previously [18]. 1–5-day- old cells were used in the experiments reported.

For capacitance measurement, recording was made using the whole-cell mode of the patch-clamp technique [19]. The external bath solution contained 145 NaCl, 5 mM KCl, 1.2 mM NaH_2PO_4, 1.3 mM $MgCl_2$, 3 mM $CaCl_2$, 10 mM glucose, 20 mM HEPES, pH 7.4. The pipette solution contained 139 mM K^+ glutamate, 2 mM ATP, 2 mM $MgCl_2$, 5 mM EGTA and 20 mM PIPES, pH 6.5, with no added $CaCl_2$ (0 Ca^{2+}) or with $CaCl_2$ added to give a free Ca^{2+} concentration of 10 μM. Where appropriate GTP analogues or other agents were included in the pipette solution. In some cases ATP was omitted from the pipette solution. Capacitance was monitored, using pipettes of resistance 3–4 MΩ in bath solution, following establishment of the whole cell recording mode using the capacitance-tracking routing of the EPC-9 patch-clamp am-

*Corresponding author. Fax: (44) (51) 794 5337.

Abbreviations: GTPγS, guanosine 5′[γ-thio]triphosphate; GppNHp, guanosine 5′-[$\beta\gamma$-imido]triphosphate; GDPβS, guanosine 5′-[β-thio]diphosphate; HEPES, 4-(2-hydroxyethyl)-1-piperazinethane sulfonic acid; Ins(1,4,5)P_3, inositol(1,4,5)trisphosphate; XTP, xanthosine triphosphate.

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plifier (HEKA, Germany). The cells were clamped at a holding junction potential of −60 mV after correction for a liquid potential of −8 mV. All experiments were carried out at room temperature (22–25°C).

3. Results and discussion

In order to compare the effect of GTP analogues on exocytosis measured by capacitance change with that on catecholamine secretion from permeabilised chromaffin cells, the pipette solution chosen for cell dialysis was the same as that we previously used for permeabilised cells [13]. To check that the cells responded as expected with this pipette solution, which differed from that previously used in patch-clamp studies [15–17] the effect of pipette buffer with no added Ca^{2+} (0 Ca^{2+}) and or with a free calcium concentration of 10 μM was examined. In the series of experiment reported here, the initial membrane capacitance was 6.1 ± 0.2 pF (n = 138). With 0 Ca^{2+} buffer the measured capacitance tended to fall slowly during the period of recording. As expected [16], internal dialysis with buffer containing 10 μM free Ca^{2+} triggered a substantial exocytotic response (in 26 out of 26 cells) seen as a large increase in capacitance usually to around double the initial capacitance level over a 10–20 min period (Fig. 1). Following an initial high rate of capacitance increase soon after the onset of internal cell dialysis, the rate of capacitance increase declined to a lower maintained rate (Fig. 1) which continued for at least 20 min. When ATP was omitted from the pipette the initial rate of capacitance increase was unaffected but the slower second phase was essentially abolished (Fig. 1b) consistent with previous work on digitonin-permeabilised cells [20]. Since capacitance increases often did not reach a plateau, quantitative comparison between cells was done by determining the maximum initial rate of capacitance change for each cell (Fig. 1b).

In our previous work using permeabilised chromaffin cells, catecholamine release could be stimulated at 0 Ca^{2+} using the non-hydrolysable analogue GppNHp but the alternative analogue GTPγS was poorly effective [13]. Consistent with these data GTPγS produced only a marginal increase in capacitance (4 cells) but a capacitance rise due to GppNHp was more consistently observed (Fig. 2). A larger stimulation of catecholamine release in permeabilised chromaffin cells was found with the GTP analogue XTP [13]. Internal dialysis with a pipette solution containing 10 mM XTP produced a marked increase in capacitance that was on average smaller in extent than that due to 10 μM Ca^{2+} (compare Figs. 1 and 2) and had a lower maximum rate of increase (Fig. 3). This difference in the effectiveness of Ca^{2+} and XTP parallels that seen in catecholamine release from permeabilised cells [13]. In a total of 18 cells with XTP in the pipette, a response similar to that shown in Fig. 2 was seen in 10 cells, 6 cells showed a slower and more prolonged

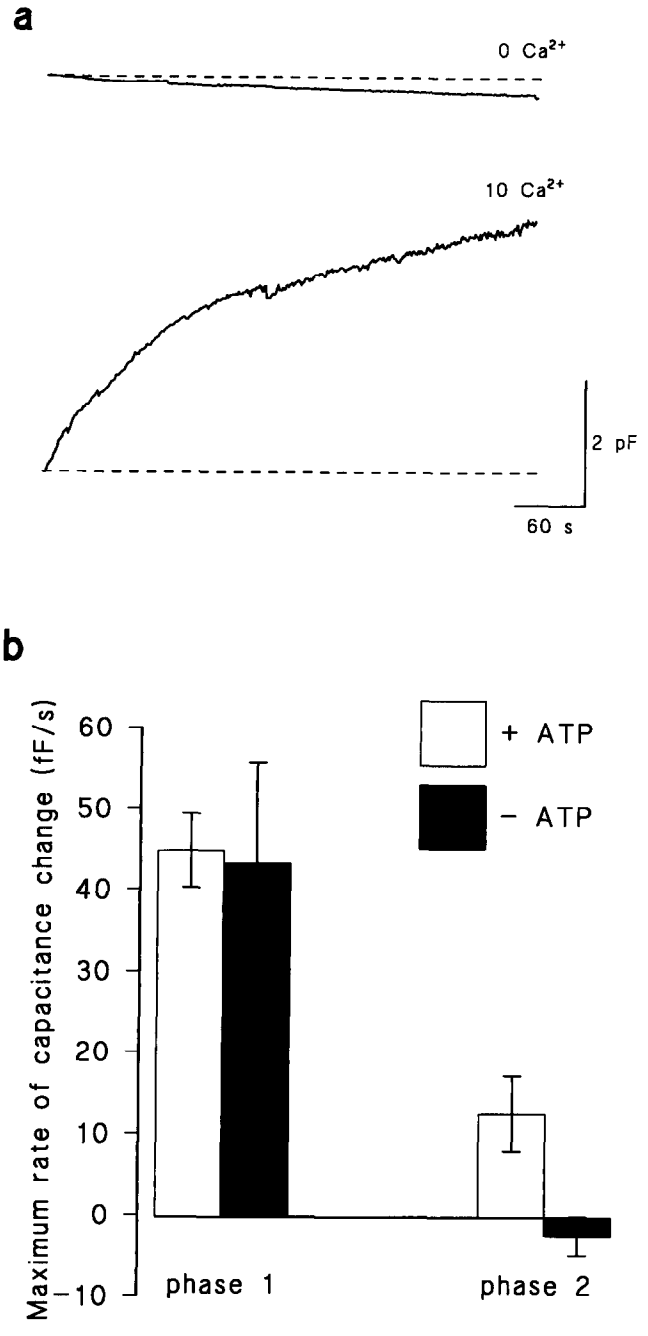

Fig. 1. Ca^{2+}-activated capacitance changes in adrenal chromaffin cells. (a) Capacitance monitored in cells dialysed with 0 Ca^{2+} or 10 μM Ca^{2+} for a 7 min period following breakthrough to the whole-cell mode. (b) Quantification of the initial maximum rate of capacitance change (phase 1) and the sustained rate of capacitance change after 300 s (phase 2) with ATP present (n = 11) or absent (n = 6) from the pipette buffer.

response and 2 cells did not respond with a capacitance increase. The maximum capacitance increase observed was in the range 1.3–3.8 pF.

To check whether XTP was acting via a GTP-binding protein, the effect of GDPβS was examined since this GTP analogue would be expected to lock GTP-binding proteins in an inactive state. GDPβS alone had no effect

on capacitance, nor on the capacitance increase due to 10 μM Ca^{2+}, but it prevented the capacitance increase due to XTP (Figs. 2 and 3). One possible mechanism of action of XTP and GppNHp in stimulating exocytosis was due to Ca^{2+} mobilisation following Ins(1,4,5)P_3 production. The 5 mM EGTA in the patch pipette giving a high level of Ca^{2+} buffering should reduce any Ca^{2+} transients due to internal Ca^{2+} mobilisation. This was confirmed by the finding that introduction of Ins(1,4,5)P_3 in the pipette buffer did not result in activation of a detectable capacitance rise (Fig. 3).

The data show that GTP analogues can activate exocytosis measured directly by the patch-clamp capacitance technique under conditions where the cytosolic Ca^{2+} concentration is clamped at low levels. The effect of the most potent analogue, XTP, appear to be mediated by a bone fide GTP-binding protein as its effect was blocked by GDPβS. The change in capacitance induced by XTP was, however, smaller than that triggered by 10 μM Ca^{2+}. These data are fully consistent with previous studies on digitonin-permeabilised chromaffin cells [13]. GDPβS had no effect on the Ca^{2+}-induced capacitance increase and so it is possible that Ca^{2+} and GTP analogues act on parallel but distinct pathways. It is unlikely that XTP is acting via a Ca^{2+}-dependent mechanism due to Ca^{2+} mobilisation since introduction of Ins(1,4,5)P_3 via the patch pipette did not result in any change in capacitance. In addition, external perfusion of the cells with caffeine which produces a marked mobilisation of intracellular Ca^{2+} [21] lead to only a small (< 0.4 pF) capacitance increase (data not shown).

With permeabilised cells, analysis of the kinetics of exocytosis is complicated by cellular heterogeneity and the fact that the measurements are dependent upon release of catecholamine from the granule core as well as exocytotic membrane fusion. The use of patch-clamp capacitance allows a clearer assessment of the kinetics and extent of exocytosis in single cells in response to GTP analogues. Recent work on the kinetics of exocytosis triggered by Ca^{2+} in chromaffin cells has shown that Ca^{2+} has two sites of action. It stimulates the rapid exocytosis of a 'release-ready' pool of granules corresponding to a capacitance increase of around 0.5 pF or fusion of 200 granules and the recruitment of a further larger pool of granules [22,23]. These data are consistent with electron microscopical observations showing only around 450 granules per cell at the cell periphery [24] and measurements on secretion from permeabilised chromaffin cells showing the presence of a limited pool of primed granules whose release is ATP-independent [20,25]. GppNHp elicited a capacitance increase similar in size to the release-ready pool. In contrast, the response to XTP, with a capacitance rise of up to 3.8 pF can not be accounted for on the basis of activation of the release-ready pool of granules and the data suggest, therefore, that GTP analogues can additionally stimulate granule recruitment. In some cells we observed that XTP lead to a capacitance rise that continued to increase for at least

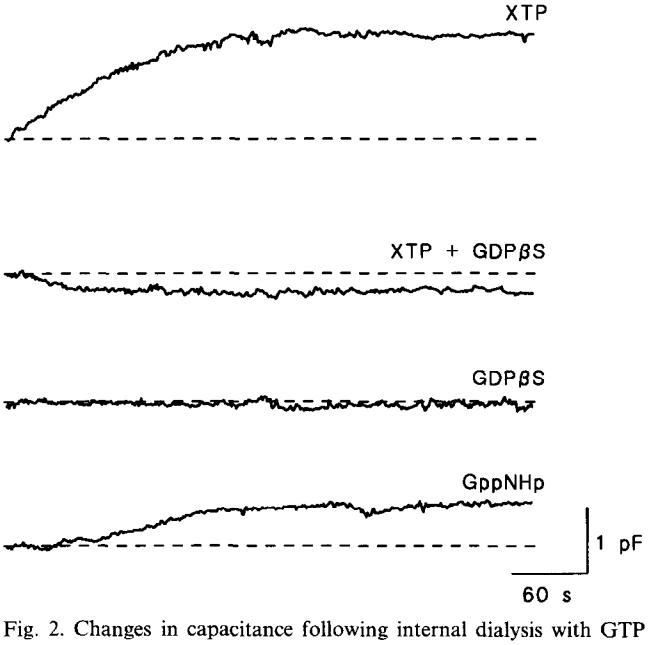

Fig. 2. Changes in capacitance following internal dialysis with GTP analogues. The pipette buffer contained no addition, 10 mM XTP, 100 μM GppNHp or 1 mM GDPβS and capacitance followed over the initial 7 min period following establishment of whole-cell recording.

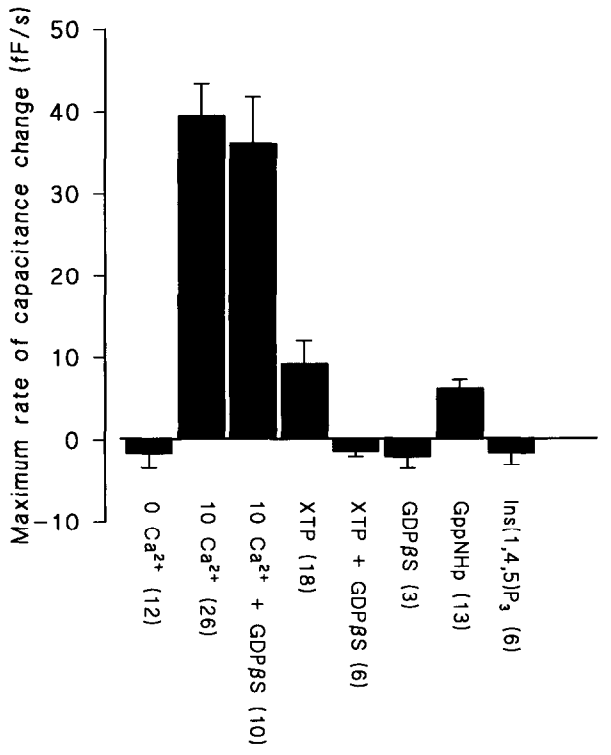

Fig. 3. Quantification of maximum rates of capacitance change in cells dialysed with various agents. The data from all of the cells in the series of experiments is shown as mean ± S.E.M. for the n values indicated. Concentrations of the added agents were: XTP, 10 mM; GDPβS, 1 mM; GppNHp, 100 μM; Ins(1,4,5)P_3, 10 μM.

15 min, consistent with this interpretation. In addition, in permeabilised chromaffin cells, GTP analogues stimulate actin reorganisation that may be required for granule recruitment [26]. Experiments with GTP analogues in permeabilised chromaffin cells [12–14,27–29] have shown a variety of effects depending on the mode of permeabilisation, the incubation time with the analogue and on the particular analogue examined. It seems likely, therefore, that multiple GTP-binding proteins exert regulatory effects on both granule recruitment and exocytosis in adrenal chromaffin cells.

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