Control of exocytosis in adrenal chromaffin cells by GTP-binding proteins studied using permeabilized cells and patch-clamp capacitance measurements

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Introduction
Studies on a variety of permeabilized secretory cell types have highlighted the important roles of Ca\(^{2+}\) and GTP-binding regulatory proteins in the control of exocytosis [1,2]. In some cell types, such as neutrophils, activation of GTP-binding proteins by non-hydrolysable GTP analogues is sufficient to activate exocytosis even at extremely low intracellular Ca\(^{2+}\) concentrations [3]. In permeabilized mast cells, GTP analogues and Ca\(^{2+}\) are needed together as dual effectors [4]. It has been suggested that in these cell types, a GTP-binding protein, \(G_{i_1}\), is a key element in the exocytotic machinery [5]. At the other end of the spectrum are cell types such as adrenal chromaffin cells, where Ca\(^{2+}\) appears to be the central trigger for exocytosis although exocytosis may be regulated by GTP-binding proteins [6]. It is not yet clear whether these cell types share the same fundamental exocytotic components, with a GTP-binding protein being essential for exocytosis in all cell types.

The aim of this article is to assess the evidence for the involvement of GTP-binding proteins in exocytosis in chromaffin cells and information on their possible nature. It appears that exocytosis in chromaffin cells is probably regulated by multiple GTP-binding proteins of both the heterotrimeric and monomeric classes, but the exact identity of these proteins has yet to be determined.

The effects of GTP analogues on exocytosis in chromaffin cells
The effects of non-hydrolysable GTP analogues (GTP\(_{i_1}\)S and GppNHp) on exocytosis in chromaffin cells permeabilized by electric discharge [7], with staphylococcal \(\alpha\)-toxin [8], streptolysin O [9,10] or digitonin [11–14] have been examined by several laboratories. The results from such experiments were initially confusing and apparently contradictory because GTP\(_{i_1}\)S inhibited Ca\(^{2+}\)-dependent secretion in electrically permeabilized cells but, in cells permeabilized by other techniques, GTP analogues either stimulated Ca\(^{2+}\)-independent secretion [11,14] or enhanced Ca\(^{2+}\)-dependent secretion [8,13]. It appeared that the differing permeabilization methods could possibly account for the three different GTP analogue responses found but it was subsequently demonstrated that the three responses could all be elicited in one study using streptolysin O-permeabilized chromaffin cells [10]. The straightforward interpretation of this set of data is that exocytosis is controlled by multiple G-proteins and that these can be differentially activated by GTP analogues under various conditions (Figure 1). This idea is reinforced by the fact that the three GTP analogue-induced responses to chromaffin cells differ in their preference for GTP\(_{i_1}\)S compared with GppNHp (Table 1).

There is one interesting difference between electroporpermeabilized and digitonin- or streptolysin O-permeabilized chromaffin cells. The inhibition by GTP\(_{i_1}\)S of Ca\(^{2+}\)-dependent secretion can only be

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*Abbreviation used: ARF, ADP-ribosylation factor.*

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revealed by preincubation with GTPyS before challenge with Ca\(^{2+}\) [7,9,12]. In electropermeabilized cells, the inhibition is almost complete [7] but is typically only a partial inhibition (30-40%) with the other permeabilization methods. One interpretation of these results is that the GTPyS-induced inhibition involves a cytosolic component (a GTP-binding protein?) that can leak from cells with large membrane pores after digitonin or streptolysin O permeabilization but does not leak through the small pores in electropermeabilized cells which are only permeable to molecules with a molecular mass of < 1000 kDa.

An increase in catecholamine release from digitonin-permeabilized cells in the absence of Ca\(^{2+}\) has been detected using a range of GTP analogues [11,14]. The most effective was XTP (10 mM) and the stimulation by this and other GTP analogues was blocked by GDP/BS [14]. One problem with permeabilized cells is that catecholamine release can occur as a result of intracellular granule lysis rather than exocytosis and, therefore, it is important to establish that stimulators do activate the exocystotic process. This can be demonstrated directly using the whole-cell patch-clamp technique to monitor membrane capacitance [15]. Measured membrane capacitance is proportional to plasma membrane area and exocytosis results in an increase in capacitance in single chromaffin cells. The cells were internally dialysed with buffer containing 5 mM EGTA but no added Ca\(^{2+}\) (0 Ca\(^{2+}\)), Ca\(^{2+}\) added to give 10 pM free Ca\(^{2+}\), or 0 Ca\(^{2+}\) with 10 mM XTP. A Ca\(^{2+}\)-dependent increase in capacitance was readily detectable (maximum rate 42.3 ± 7.3 fF/s, mean ± S.E.M., n = 12 cells). In addition, in 0 Ca\(^{2+}\), XTP stimulated exocytosis (maximum rate 9.5 ± 3.9 fF/s, n = 12, compared with 0.36 ± 0.53 fF/s, n = 8, in the absence of XTP) but the responses fell into two groups with either slow (n = 8) or fast (n = 4) responses (Figure 2). These data confirm that exocytosis can be activated in chromaffin cells at very low Ca\(^{2+}\) concentration by GTP analogues.

### Evidence for the involvement of heterotrimeric G-proteins

Treatment of chromaffin cells with pertussis toxin results in an enhancement of secretion from intact and permeabilized cells, suggesting that exocytosis is tonically inhibited by a pertussis toxin-sensitive heterotrimeric G-protein [16-18]. This phenomenon was explored in more detail by the labora-

![Figure 1](image)

**Figure 1**

**Effect of GTP\(^{\gamma}\)S on secretion from digitonin-permeabilized chromaffin cells**

Chromaffin cells were permeabilized with digitonin for 10 min [14] in the presence or absence of 100 µM GTPyS and then incubated for a further 20 min with or without GTPyS in 0.3 or 10 µM free Ca\(^{2+}\). At the end of this incubation, released catecholamine was assayed and expressed (mean ± S.E.M., n = 3) as a percentage of total cellular catecholamine. GTPyS enhances release at 300 nM Ca\(^{2+}\) but partially inhibits release at 10 µM Ca\(^{2+}\).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Catecholamine released (%)</th>
</tr>
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<tbody>
<tr>
<td>300 nM Ca(^{2+})</td>
<td>25</td>
</tr>
<tr>
<td>10 µM Ca(^{2+})</td>
<td>0</td>
</tr>
</tbody>
</table>

### Table 1

**Effects of GTP analogues on secretion from bovine adrenal chromaffin cells**

The permeabilization techniques used α-toxin (A), digitonin (D), electroperrmeabilization (E) or streptolysin O (S). Data taken from [7-14].

<table>
<thead>
<tr>
<th>Response</th>
<th>Potency of GTP analogue</th>
<th>Permeabilization technique</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stimulation of Ca(^{2+})-independent release</td>
<td>GppNHp &gt; GTP(^{\gamma})S</td>
<td>D, S</td>
</tr>
<tr>
<td>Enhancement of Ca(^{2+})-dependent release</td>
<td>GppNHp = GTP(^{\gamma})S</td>
<td>A, D, S</td>
</tr>
<tr>
<td>Inhibition of Ca(^{2+})-dependent release</td>
<td>GTP(^{\gamma})S &gt; GppNHp</td>
<td>A, D, E, S</td>
</tr>
</tbody>
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**Effect of Ca²⁺ and XTP on exocytosis in chromaffin cells monitored by patch-clamp capacitance**

Chromaffin cells were internally dialysed with buffer (137 mM K glutamate, 2 mM ATP, 2 mM MgCl₂, 5 mM EGTA, 20 mM Pipes, pH 6.5) using the whole-cell patch-clamp technique and capacitance was monitored using the capacitance-tracking routine of the EPC-9 amplifier. The buffer contained: (a) no additions to give 0 Ca²⁺; (b) added CaCl₂ to 10 μM free Ca²⁺; or (c,d) 10 mM XTP at 0 Ca²⁺. The two traces shown for XTP demonstrate the two types of kinetic response found. The values of initial cell capacitance are shown on the right of each trace.

(a) 6.3

(b) 3.4

(c) 3.9

(d) 4.8

60 s

2 pF

**Conclusions**

In the adrenal chromaffin cell, where Ca²⁺ is the key trigger for exocytosis, GTP-binding proteins appear to control the exocytotic process. Inhibitory and stimulatory control is exerted apparently through both heterotrimeric and monomeric GTP-binding proteins.
binding proteins. Further work will be required to determine exactly which GTP-binding proteins are involved, whether they also function in an essential manner in the exocytotic fusion machinery or in its assembly, and to find out if they act on vesicle docking, fusion or both. The ability of GTP analogues to directly stimulate Ca\(^{2+}\)-independent exocytosis is consistent with a key role for a GTP-binding protein (G, \([5]\)) in the triggering of exocytosis.

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