

# Neomycin Is a Potent Secretagogue of Mast Cells That Directly Activates a GTP-binding Protein Involved in Exocytosis

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**Abstract.** When loaded alongside GTP- $\gamma$ -S into ATP-permeabilized cells, neomycin, at concentrations below 1 mM, inhibits GTP- $\gamma$ -S-induced histamine secretion and phosphatidic acid formation (Cockcroft, S., and B. D. Gomperts, 1985. *Nature (Lond.)*. 314: 534-536; Aridor, M., L. M. Traub, and R. Sagi-Eisenberg. 1990. *J. Cell Biol.* 111:909-917). However, at higher concentrations internally applied neomycin induces histamine secretion in a process that is: (a) dose dependent; (b) dependent on the internal application of GTP; (c) independent of phosphoinositide breakdown; and (d) inhibited by pertussis toxin (PtX) treatment. These results indicate that neomycin can stimulate histamine secretion in a mechanism that bypasses phospholipase C (PLC) activation and yet involves a PtX-sensitive GTP-binding protein (G protein). Unlike its dual effects, when internally applied, neomycin induces histamine secretion from intact mast cells in a dose-dependent manner. Half-maximal and maximal

effects are obtained at 0.5 and 1 mM neomycin, respectively. This process is rapid ( $\sim 30$  s), is independent of external  $\text{Ca}^{2+}$ , and is associated with phosphatidic acid formation, implying that neomycin can activate histamine secretion by a mechanism similar to that utilized by other basic secretagogues of mast cells. Neomycin stimulates fourfold the GTPase activity of cholerae-solubilized rat brain membranes in a PtX-inhibitable manner. In addition neomycin, as well as the basic secretagogues of mast cells, compound 48/80, and mastoparan, significantly reduce (by  $\sim 80\%$ ) the ADP ribosylation of PtX substrates present in rat brain membranes. Taken together these data suggest that neomycin can stimulate secretion from mast cells by directly activating G proteins that play a role in stimulus-secretion coupling. When internally applied, neomycin presumably stimulates secretion by activating a G protein that is located downstream to PLC. This G protein serves as a substrate for PtX.

HISTAMINE secretion from rat peritoneal mast cells can be triggered by the introduction of nonhydrolyzable analogues of GTP, such as GTP- $\gamma$ -S, into cells permeabilized with ATP (Gomperts, 1983). Under these conditions secretion is associated with phosphatidic acid (PA)<sup>1</sup> formation, and both processes, histamine secretion (Cockcroft and Gomperts, 1985) and PA formation (Aridor et al., 1990), are inhibited by the introduction of the positively charged aminoglycoside antibiotic neomycin into the ATP-permeabilized cells. Neomycin is known to bind strongly and selectively to inositol phospholipids (Schacht, 1978), thereby leading to inhibition of their metabolism. It therefore appears that the persistent activation of a G protein that is coupled to a phosphoinositide-hydrolyzing phospholipase C (PLC) ( $G_p$ ) brings about histamine secretion. However, GTP and its analogues do not affect the activity of  $G_p$  exclusively. Mast cells, permeabilized with streptolysin-O, which generates membrane lesions permitting the efflux of cytosolic components, are capable of responding to GTP- $\gamma$ -S

and secreting histamine also in the presence of neomycin (Howell et al., 1987). Thus, the activation of a G protein involved at a late stage in the stimulus-secretion process ( $G_E$ ) seems to provide a sufficient signal for exocytosis.

Histamine secretion from rat peritoneal mast cells can be activated by a family of basic compounds such as compound 48/80 (Ennis et al., 1980), substance P (Fewtrell et al., 1982), and histone (Sagi-Eisenberg et al., 1985). We have demonstrated (Aridor et al., 1990) that the basic secretagogues, compound 48/80 and mastoparan, are capable of stimulating secretion from ATP-permeabilized cells in a process that is not accompanied by PA formation but is dependent on the internal application of GTP. We could further demonstrate that compound 48/80, like mastoparan (Higashijima et al., 1988), is capable of directly stimulating the GTPase activity of G proteins in a cell-free system. Based on these results, we proposed that basic secretagogues of mast cells may directly activate a G protein that is located downstream from PLC ( $G_E$ ), thereby inducing secretion in a mechanism that bypasses PLC activation.

Neomycin has previously been shown to manifest biological effects on cells, in addition to its known inhibitory action on phosphoinositide metabolism. For example, depending

1. *Abbreviations used in this paper:* PA, phosphatidic acid; PLC, phospholipase C; PtX, pertussis toxin.

on its concentration neomycin was shown to stimulate secretion, aggregation responses, and arachidonic acid release from semi-permeabilized platelets (Nakashima et al., 1987; Polascik et al., 1987). Neomycin was also shown to modify the activity of human platelet membrane GTPase (Hermann and Jakobs, 1988) and to induce high-affinity agonist binding of G protein-coupled receptors (Hermann et al., 1989). Therefore, to further investigate our hypothesis, in this study we have examined whether neomycin may activate secretion from mast cells in a mechanism that is independent of phosphoinositide breakdown. Indeed, we show that neomycin acts as a potent secretagogue of mast cells by directly activating G proteins involved in exocytosis.

## Materials and Methods

### Materials

[<sup>32</sup>P]Orthophosphate (9,400 Ci/mmol) was purchased from the Nuclear Center (Negev, Israel). [<sup>32</sup>P]GTP (3,000 Ci/mmol) and [<sup>32</sup>P]NAD (1 μCi) and 1 μg of preactivated PtX. Reaction was stopped by adding concentrated Laemmli sample buffer. Samples were analyzed by SDS-PAGE and autoradiography of the gels.

### Cells

Mast cells from the peritoneal cavity of male Wistar rats (250–300 g body weight) were isolated and purified over a Ficoll gradient as previously described (Sagi-Eisenberg et al., 1983). Purity of the cell population was determined using toluidine blue staining and cell viability was determined using trypan blue exclusion. Under these conditions a homogeneous mast cell population was obtained (>90%) with 95% viability.

### Cell Permeabilization

Purified mast cells were permeabilized as described in Aridor et al. (1990).

### Histamine Secretion

Histamine secretion was measured as previously described (Sagi-Eisenberg et al., 1983).

### Phospholipid Analysis

Analysis of phospholipids was carried out as described in Aridor et al. (1990).

### Membrane Preparation

Rat brain membranes were prepared according to Katada et al. (1986).

### GTPase Assay

The GTPase assay was performed according to Gibbs et al. (1988). 30-μg aliquots of cholate solubilized rat brain membranes were incubated in the presence of the indicated reagents for 5 min at 25°C in a final volume of 100 μl. The reaction mixture contained final concentrations of 0.1 mM ATP, 0.2 mM ATP-γ-S, 0.3 μM GTP, 0.1 mM EGTA, 1 mM DTT, 2 mM MgAc<sub>2</sub>, 5 mM phosphocreatine, 0.4 mg/ml creatine kinase, 20 mM Tris-HCl, pH 7.4, and 0.1 μCi of [<sup>32</sup>P]GTP. Reaction was stopped by spotting 5-μl aliquots onto poly(ethylene)imine-cellulose TLC plates. The plates were developed with 1 M KH<sub>2</sub>PO<sub>4</sub>, pH 4.5, and visualized by autoradiography. The spots corresponding to GDP were analyzed by Molecular Dynamics Computing Densitometry (Sunnyvale, CA). Specific GTPase activity was calculated from the difference between [<sup>32</sup>P]GDP released in the absence or presence of 100 μM GTP.

### ADP Ribosylation

Cholate-solubilized rat brain membranes (80 μg) were incubated for 30 min at 30°C in the presence of 1 mM ATP, 15 mM thymidine, 100 mM potas-

sium phosphate buffer, pH 7.5, 3 mM DTT, 20 μM [<sup>32</sup>P]NAD (1 μCi), and 1 μg of preactivated PtX. Reaction was stopped by adding concentrated Laemmli sample buffer. Samples were analyzed by SDS-PAGE and autoradiography of the gels.

### Presentation of Data

The data points presented are means of duplicate determinations that did not vary by more than 2%. Similar results were obtained on at least two occasions.

## Results

### Effect of Neomycin on Histamine Secretion from Intact Mast Cells

Addition of neomycin at concentrations >0.4 mM to intact, purified rat peritoneal mast cells resulted in their degranulation as evidenced by the secretion of the two granular components histamine (Fig. 1 A) and the enzyme β-hexosaminidase (not shown). A concentration of 1 mM neomycin stimulated secretion of 50% of the granular content, while a half-maximal effect was achieved at a concentration of 0.5 mM neomycin (Fig. 1 A).

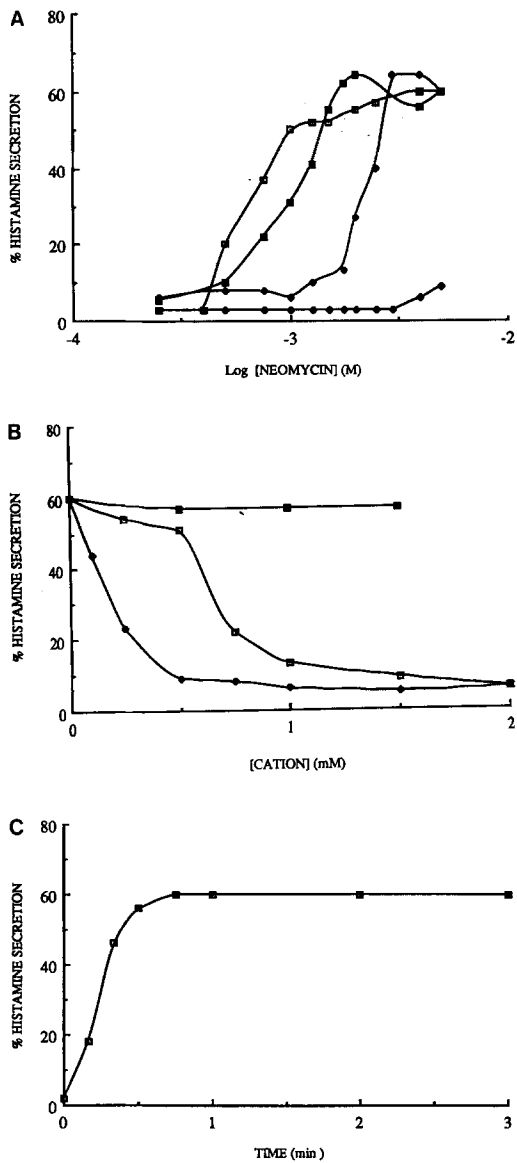
Histamine secretion induced by neomycin did not require the presence of exogenous Ca<sup>2+</sup>. In fact, as previously found for other positively charged secretagogues such as compound 48/80 (Atkinson et al., 1979) and substance P (Fewtrell et al., 1982), Ca<sup>2+</sup> antagonized neomycin-induced secretion and this inhibitory effect could only be overcome in the presence of supermaximal concentrations of neomycin (Fig. 1 A). Mg<sup>2+</sup> also had a small inhibitory effect on the induced release. However, this effect was significant only at submaximal concentrations of neomycin (Fig. 1 A). The most pronounced effect was exerted by Mn<sup>2+</sup>, which completely abolished neomycin-induced secretion (Fig. 1 A).

Both Ca<sup>2+</sup> and Mn<sup>2+</sup> inhibited secretion induced by an optimal concentration of neomycin (1.5 mM) in a dose-dependent manner (Fig. 1 B). Maximal inhibition (90%) was obtained at concentrations of 1 mM Mn<sup>2+</sup> and 2 mM Ca<sup>2+</sup>, while half-maximal inhibition was exerted by concentrations of 0.2 mM Mn<sup>2+</sup> and 0.7 mM Ca<sup>2+</sup> (Fig. 1 B). At this neomycin concentration Mg<sup>2+</sup> had no inhibitory effect (Fig. 1 B).

Neomycin-induced secretion was rapid and virtually complete within 30 s of adding neomycin (Fig. 1 C). The release showed similar kinetics when measured at different neomycin concentrations (not shown).

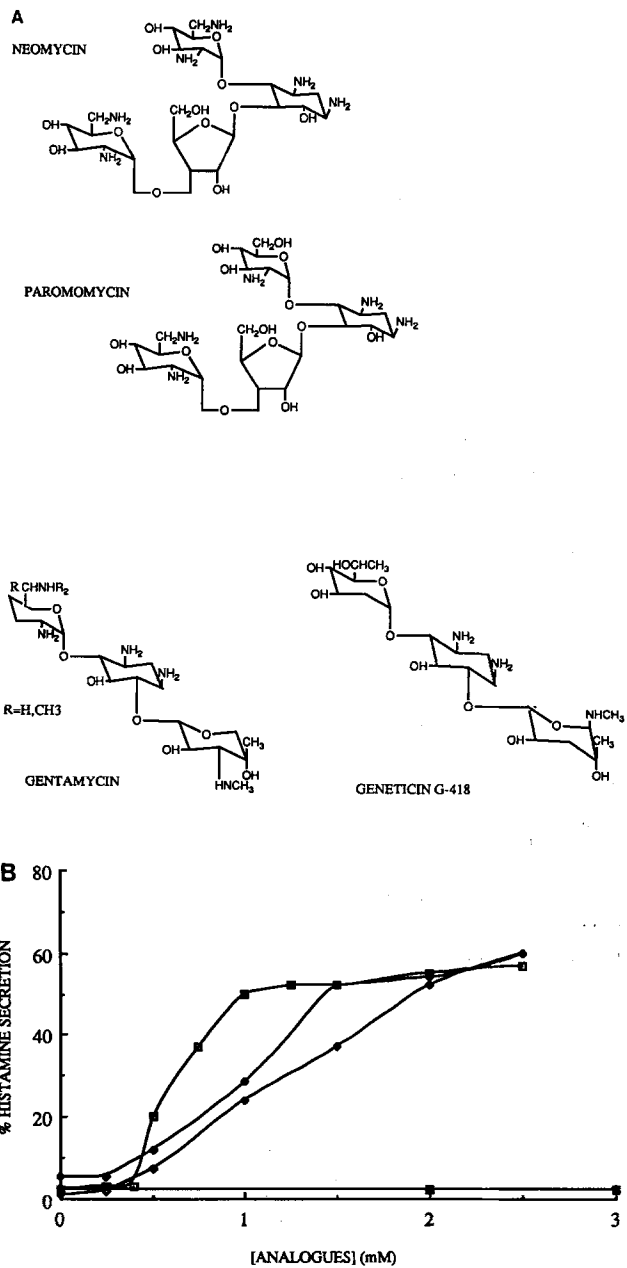
### Effect of Neomycin Analogues on Histamine Secretion from Intact Mast Cells

Three other aminoglycoside antibiotics, related to neomycin, were also tested for their ability to induce secretion from intact mast cells. Gentamycin and paromomycin, which include five of the six amino groups present in neomycin (Fig. 2 A) stimulated secretion to a similar extent (60%) in a dose-dependent fashion (Fig. 2 B). Nevertheless, while comparable in their relative potencies, both gentamycin and paromomycin were less effective than neomycin when compared on a molar basis. Their maximal effects were achieved at concentrations of 1.5 and 2 mM and their half-maximal effects at 1.0 and 1.2 mM gentamycin and paromomycin, respectively. Another analogue of neomycin, geneticin (G-418), which contains only three amino groups (Fig. 2 A), was com-



**Figure 1.** (A) Neomycin-induced histamine secretion from intact mast cells. Mast cells were incubated for 20 min at 30°C with the indicated concentrations of neomycin in the absence (□) or presence of 2.5 mM Mg<sup>2+</sup> (■), Ca<sup>2+</sup> (◆), and Mn<sup>2+</sup> (●). The reaction was stopped and histamine secretion was determined as described in Materials and Methods. (B) Effect of divalent cations on neomycin-induced histamine secretion. Mast cells were incubated for 20 min at 30°C in the presence of 1.5 mM neomycin and the indicated concentrations of Mn<sup>2+</sup> (◆), Ca<sup>2+</sup> (□), and Mg<sup>2+</sup> (■). The reaction was stopped and histamine secretion was determined as described in Materials and Methods. (C) Kinetics of neomycin-induced histamine secretion. Mast cells were incubated with 5 mM neomycin for the indicated time periods. Reaction was stopped and histamine secretion was determined as described in Materials and Methods.

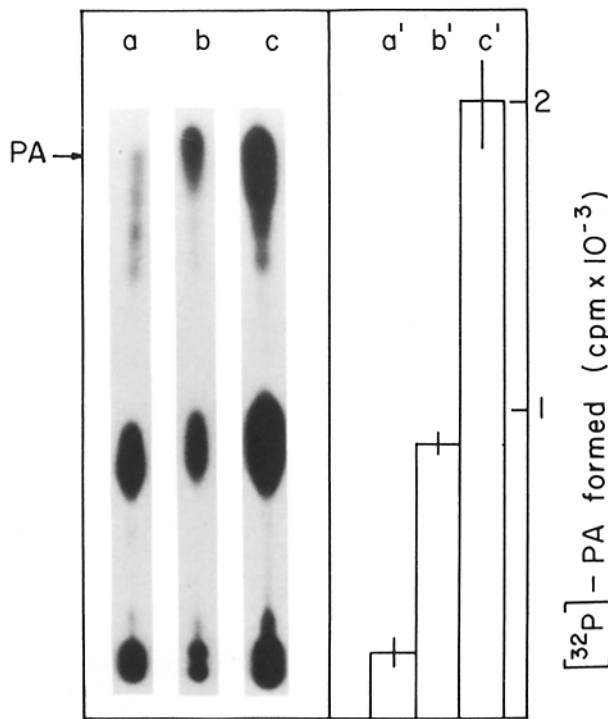
pletely ineffective and failed to induce secretion (Fig. 2 B) even when tested at concentrations up to 7 mM (not shown). Hence, a correlation seems to exist between the positive charge of the agonist tested and its potency in inducing the secretory response.



**Figure 2.** (A) Structure of neomycin analogues. (B) Effect of neomycin analogues on histamine secretion. Mast cells were incubated for 20 min at 30°C with the indicated concentrations of neomycin (□), gentamycin (◆), paromomycin (◇), and geneticin G-418 (■). The reaction was stopped and histamine secretion was determined as described in Materials and Methods.

### Effect of Neomycin on PA Formation

Histamine secretion induced by neomycin involved the activation of a phospholipase leading to PA formation. This is illustrated in Fig. 3, where, similarly to the effect of compound 48/80 that stimulated eightfold PA formation (lanes c and c', compared with a and a'), the addition of 5 mM neomycin to <sup>32</sup>P-labeled cells resulted in 3.6-fold enhancement of the formation of [<sup>32</sup>P]PA (lanes b and b', compared with a and a'). This increase in PA probably reflected a rise in di-



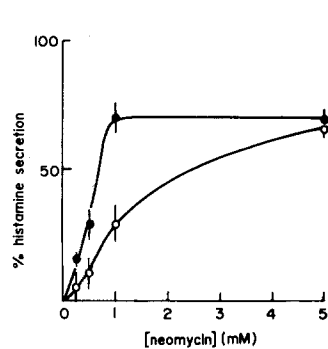
**Figure 3.** Effect of neomycin on PA formation by intact mast cells. Purified mast cells ( $1 \times 10^6$  cells/ml) were incubated for 2 h at  $30^\circ\text{C}$  with  $^{32}\text{P}$ i (100  $\mu\text{Ci}/\text{ml}$ ). Cells were subsequently washed and incubated for 20 min at  $30^\circ\text{C}$  in a  $\text{Ca}^{2+}$ -containing buffer in the absence (lane *a*) or presence of neomycin (5 mM; lane *b*) or 48/80 (10  $\mu\text{g}/\text{ml}$ ; lane *c*). Reaction was stopped and PA formed was determined as described in Materials and Methods. The spots corresponding to PA were cut and counted by Cernekov (lanes *a'*-*c'*, respectively). The results of a representative experiment are presented. Similar results were obtained on three occasions.

acylglycerol levels due to a stimulated metabolism of inositol phospholipids. This is suggested by the finding that PA formation was not stimulated in neomycin-treated cells into which neomycin had been introduced by ATP permeabilization.

These results, therefore, indicate that when added to intact cells neomycin interacts with a membrane component, which eventually activates a PLC leading to phosphoinositide breakdown and histamine secretion. In contrast, when loaded into the cells neomycin binds to the inositol phospholipids present in the inner leaflet of the membrane, thereby causing the inhibition of phosphoinositide metabolism.

#### Effect of Neomycin on Histamine Secretion from ATP-permeabilized Cells

We have previously shown (Aridor et al., 1990) that compound 48/80 can trigger secretion from ATP-permeabilized and resealed mast cells in a process that is independent of PLC activation, but requires the internal application of GTP. To determine whether neomycin could act in a similar manner, histamine secretion was studied in cells loaded with neomycin under conditions that completely block PLC activation. As illustrated in Fig. 4, neomycin introduced into



**Figure 4.** Effect of GTP on neomycin-induced histamine secretion from permeabilized mast cells. Purified mast cells were permeabilized as described in Materials and Methods in the presence of the indicated concentrations of neomycin and the presence (●) or absence (○) of 1 mM GTP. Cells were subsequently resealed and histamine secretion was determined.

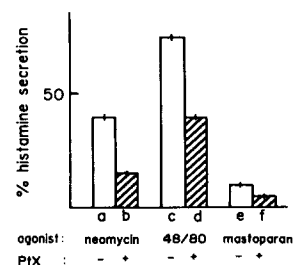
ATP-permeabilized mast cells was still capable of stimulating histamine secretion in a mechanism that was independent of phosphoinositide metabolism. However, unlike the release from intact cells (Fig. 1 *A*), higher concentrations of neomycin were required to elicit secretion from ATP-permeabilized cells.

The dose-response curve for neomycin could be shifted leftwards by introducing 1 mM GTP into the cells during their permeabilization. In the presence of GTP the effective concentration of neomycin required to induce secretion was similar to that of intact cells, and maximal secretion was obtained with 1 mM neomycin (Fig. 4). This result, therefore, indicates that diminution of intracellular GTP inhibits the cell's responsiveness to neomycin treatment.

#### Effect of PtX on Histamine Secretion Induced by Loaded Neomycin

The dependency of loaded neomycin on the presence of intracellular GTP to elicit secretion suggests that the internally applied neomycin can trigger secretion by activating a G protein that is located at a step distal to PLC. A plausible candidate is the previously suggested  $G_E$  protein (Barrowman et al., 1986). To determine whether  $G_E$  is a PtX substrate, we have analyzed the sensitivity of histamine release, induced by loaded neomycin, to PtX treatment.

As shown in Fig. 5, release induced by entrapped neomycin retained its sensitivity to PtX treatment. Cells that were ATP-permeabilized and loaded with 1 mM neomycin after 2 h of treatment with 100 ng/ml PtX secreted only 40% of



**Figure 5.** Effect of PtX on neomycin-induced histamine secretion from permeabilized cells. Purified mast cells ( $1 \times 10^6$  cells/ml) were incubated for 2 h at  $30^\circ\text{C}$  with (b, d, f) or without (a, c, e) PtX (100 ng/ml). The cells were washed and permeabilized for 6 min in the presence (a, b) or absence (c-f) of 1 mM neomycin.

The cells were subsequently resealed and further incubated for 20 min with compound 48/80 (5  $\mu\text{g}/\text{ml}$ ) (c, d), or mastoparan (100  $\mu\text{M}$ ) (e, f). The reaction was stopped and histamine secretion was determined. The mean values of duplicate determinations  $\pm$ SE are presented. Similar results were obtained on two occasions.

the histamine secreted from nontreated cells (Fig. 5, lanes *a* and *b*). Under the same conditions the cell responsiveness to two other basic secretagogues, compound 48/80 (5  $\mu\text{g}/\text{ml}$ ; Fig. 5, lanes *c* and *d*) and mastoparan (100  $\mu\text{M}$ ; Fig. 5, lanes *e* and *f*) was lowered by a similar degree. These results, therefore, suggest that  $G_E$  is a PtX substrate.

### Effect of Neomycin on GTPase Activity of Rat Brain Membranes

We have previously shown (Aridor et al., 1990) that compound 48/80, like mastoparan (Higashijima et al., 1988), can stimulate the GTPase activity of cholate-solubilized rat brain membranes. We have, therefore, examined whether neomycin can also stimulate GTPase activity under these conditions. Neomycin, like mastoparan, stimulated three- to fourfold the GTPase activity of cholate-solubilized rat brain membranes (Table I). Furthermore, this stimulatory effect was significantly reduced when the membranes were subjected to ADP ribosylation by PtX before the GTPase assay (Table I). These results, therefore, indicate that neomycin is capable of directly activating G proteins.

### Effect of Basic Secretagogues on PtX-catalyzed ADP Ribosylation

PtX-catalyzed ADP-ribosylation of G protein  $\alpha$ -subunits reflects the state of activation of the G protein. Since it is only the inactive, holo G protein that serves as a substrate for ADP ribosylation by PtX, we examined the ability of the basic secretagogues mastoparan, neomycin, and compound 48/80 to alter ADP ribosylation.

PtX catalyzed the ADP ribosylation of proteins in the 40-kD range (Fig. 6, lanes *b* and *c* compared with *a*). Despite the apparent homogeneity of the PtX-labeled band on SDS-PAGE, this band represents several substrates for PtX that are present in rat brain membranes. Including 100  $\mu\text{M}$  mastoparan (Fig. 6, lane *d*), 2.5 or 5 mM neomycin (lanes *e* and *f*, respectively); or 0.5, 1, or 2  $\mu\text{g}/\text{ml}$  compound 48/80 (lanes *g*, *h*, and *i*, respectively) in the reaction mixture resulted in decreased ADP ribosylation. At a concentration of 5 mM neomycin, ribosylation was inhibited  $\sim 80\%$  (Fig. 6, lane *f* compared with lanes *b* and *c*). These results, therefore, further suggest that these basic secretagogues enhanced subunit dissociation and activation of G proteins.

### Discussion

Previous studies have indicated the involvement of a GTP-

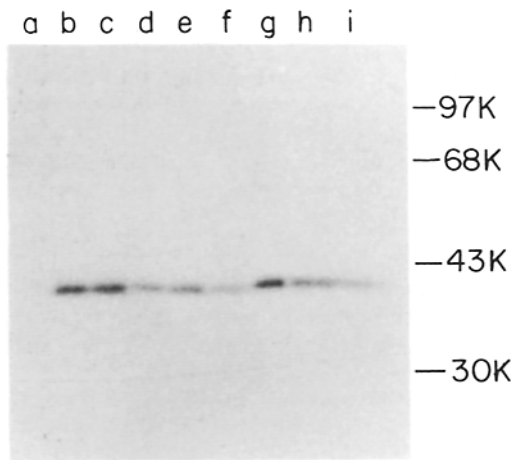


Figure 6. Effect of basic secretagogues of mast cells on PtX-catalyzed ADP ribosylation. Cholate-solubilized rat brain membranes (80- $\mu\text{g}$  aliquots) were ADP ribosylated in the absence (*a*) or presence (*b*-*i*) of activated PtX (10  $\mu\text{g}/\text{ml}$ ) and the following reagents: none (*b*, *c*), 100  $\mu\text{M}$  mastoparan (*d*), 2.5 and 5 mM neomycin (*e* and *f*, respectively), 0.5, 1, and 2,  $\mu\text{g}/\text{ml}$  compound 48/80 (*g*, *h*, and *i*, respectively). The results of a representative experiment are presented. Similar results were obtained on four occasions.

binding protein ( $G_E$ ), located distal to PLC, in exocytosis of mast cells (Howell et al., 1987). It appears that  $G_E$  is normally inhibited by an as yet unknown inhibitory factor. Unless activated either by the dialysis of this factor in patch clamped cells (Fernandez et al., 1984) or by some physiological, yet unidentified modulators,  $G_E$  fails to bind GTP or GTP- $\gamma$ -S and activate exocytosis.

We have previously shown (Aridor et al., 1990) that the basic secretagogues, compound 48/80 and mastoparan, can stimulate secretion in a mechanism that bypasses PLC activation but involves activation of a G protein. We (Aridor et al., 1990) and others (Mousli et al., 1990) could further demonstrate that compound 48/80, like mastoparan (Higashijima et al., 1988), can directly stimulate the GTPase activity of G proteins. Based on these and other studies (Repke et al., 1987), we suggested that molecules whose structure involves positive charges associated with a hydrophobic domain and that can be inserted into the lipid bilayer (mastoparan, compound 48/80, substance P), or a high density of

Table I. Activation of GTPase Activity

	[ <sup>32</sup> P]GDP formed* (arbitrary units)		
	Experiment I	Experiment II	
	Control membranes <sup>‡</sup>	Control membranes	PtX-treated membranes
None	1,489 ± 85	1,500 ± 100	1,500 ± 500
Neomycin (5 mM)	5,500 ± 500	7,000 ± 530	3,300 ± 825
Mastoparan (100 $\mu\text{M}$ )	2,570 ± 390	ND	ND

Rat brain membranes (4.5 mg/ml) were solubilized with 1% cholate and incubated for 1 h at 30°C with the ADP ribosylation reaction mixture described in Materials and Methods in the absence (control membranes) or presence (PtX-treated) of activated PtX (5  $\mu\text{g}$ ). Aliquots (30  $\mu\text{g}$ ) were then added to the GTPase reaction mixture and the GTPase activity was determined as described in Materials and Methods.

\* Spots corresponding to [<sup>32</sup>P]GDP formed were analyzed by Molecular Dynamics Computing densitometry. Values obtained in the presence of 0.1 mM GTP were subtracted.

‡ The results presented are means of duplicate determinations  $\pm$ SE of two representative experiments. Similar results were obtained on six occasions.

positive charges (poly-lysine, histone) that may disrupt membrane structure (Sande and Mandell, 1985), may activate secretion in a receptor-independent manner. This secretion is mediated by directly activating G proteins. Therefore, by directly activating the putative  $G_E$  protein these agents may stimulate secretion that is independent of phosphoinositide breakdown. In this study we have further challenged this hypothesis by testing the ability of neomycin to stimulate secretion. We postulated that this positively charged aminoglycoside may activate secretion in a mechanism that is similar to that utilized by other polybasic molecules. Indeed, here we demonstrate that neomycin added to intact cells induces their degranulation in a process that is rapid, does not require the presence of external  $Ca^{2+}$ , and is associated with phosphoinositide breakdown (Figs. 1 and 3). Based on these criteria, it appears that neomycin and the previously reported family of basic secretagogues of mast cells release histamine by a common mechanism.

Our results further demonstrate that caution should be taken when using neomycin as a selective inhibitor of phosphoinositide hydrolysis (Siess and Lapetina, 1986; Nakashima et al., 1987; Polascik et al., 1987; Hermann et al., 1989). When added to intact cells, neomycin can clearly interact with a membrane component that eventually activates phosphoinositide breakdown and histamine secretion. This membrane component could be a receptor for polybasic secretagogues. However, since receptors for polybasic secretagogues have not been identified yet, while basic secretagogues such as mastoparan (Higashijima et al., 1988), compound 48/80 (Aridor et al., 1990; Mousli et al., 1990), and neomycin (Hermann and Jakobs, 1988) (Table I) stimulate GTPase activity of G proteins, these data strongly suggest that this class of secretagogues triggers secretion by directly activating G proteins that play a role in the stimulus-secretion coupling.

Sialic acid residues or acidic phospholipids are probably the putative targets for binding these polybasic secretagogues (Shibata et al., 1984, 1985). After their primary interaction with negatively charged sites in the membrane, these basic secretagogues might be inserted into the membrane, allowing the activation of PtX-sensitive G proteins (Wakamatsu et al., 1983). The degree of amphiphilicity of a certain secretagogue will thus be an important factor in defining its specific interaction with the membrane and the extent of its insertion into the membrane (Sargent and Schwyzer, 1986). The somewhat slower rate of histamine secretion induced by neomycin ( $\sim 30$  s; Fig. 1 C) when compared with secretion induced by 48/80 or mastoparan ( $\sim 10$  s) may reflect the lower amphiphilicity of neomycin. Further support to this notion comes from the observation that the potency of neomycin and three related analogues (gentamycin, paromomycin, and geneticin G-418) in stimulating the secretory response is correlated with the degree of their cationic charges (Fig. 2). In addition, divalent cations that are known to bind acidic phospholipids inhibit the neomycin-induced response, probably by competing with neomycin for binding sites (Fig. 1 A). Moreover, the effectiveness of the divalent cations in inhibiting neomycin-induced response (Fig. 1 B) is well correlated with their effectiveness in inducing aggregation of phosphatidylserine vesicles ( $Mn^{2+} > Ca^{2+} > Mg^{2+}$ ) (Ohki et al., 1982).

The interaction between basic secretagogues and G pro-

teins is further illustrated in their ability to inhibit PtX-catalyzed ADP ribosylation. Mastoparan, compound 48/80, and neomycin all inhibited ADP ribosylation of rat brain membrane proteins (Fig. 6). This altered susceptibility of the G proteins to ADP ribosylation by PtX reflects a conformational shift that is associated with their activation.

In the presence of internally loaded neomycin (introduced into ATP-permeabilized cells), phosphoinositide hydrolysis is inhibited. This inhibition probably reflects the known action of neomycin that binds to inositol phospholipids, thereby inhibiting their metabolism (Schacht, 1978). Nevertheless, under these conditions neomycin is still capable of stimulating secretion, provided that either its concentration is increased to 1 mM (or above), or that 1 mM GTP is also entrapped in the ATP-permeabilized cells (Fig. 4). Hence, while neomycin, loaded at low concentrations ( $<1$  mM), inhibits phosphoinositide breakdown and secretion that is dependent on PLC activation (Cockcroft and Gomperts, 1985), at higher concentrations and in the presence of 1 mM GTP neomycin can trigger secretion by activating a G protein ( $G_E$ ) that is located downstream from PLC.

Together our findings imply that neomycin can interact with more than one G protein in the signal transduction cascade. When applied to intact cells neomycin may activate  $G_P$  and possibly  $G_E$  to stimulate secretion that is associated with phosphoinositide breakdown. When applied internally neomycin self-inhibits phosphoinositide metabolism, therefore leading to secretion that is mediated by  $G_E$  only. The finding that under these conditions PtX treatment inhibits secretion (Fig. 5) indicates that  $G_E$  is a substrate for ADP ribosylation by PtX.

In conclusion, basic secretagogues, such as neomycin and mastoparan, that block phosphoinositide breakdown by binding to these negatively charged phospholipids (Wojcikiewicz and Nahorski, 1989), can be used as a tool to study the cascade of events involved in  $G_E$ -activated histamine secretion.

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