Previously we observed that rab3 GTPases modulate both the secretion of catecholamines from PC12 neuroendocrine cells and the steady-state accumulation of exogenous norepinephrine (NE) into these cells (Weber, E., Jilling, T., and Kirk, K. L. (1996) J. Biol. Chem. 271, 6963–6971). Here we addressed the mechanisms by which these monomeric GTPases stimulate NE uptake by PC12 cells including their effects on uptake kinetics, their sites of action (secretory granule membrane versus plasma membrane), and the involvement of rab3-interacting proteins in this process. We observed that rab3B stimulated the rate and maximal accumulation of radio-labeled NE into large dense core vesicles within intact PC12 cells. rab3A and rab3B also increased NE uptake into large dense core vesicles in digitonin-permeabilized PC12 cells, which indicates that these GTPases stimulate catecholamine uptake at the level of the secretory granule membrane. In an attempt to identify rab3B targets that may mediate this effect on NE uptake, we found that rab3B interacts directly with phosphoinositide 3-kinase (PI3K) in a GTP-dependent fashion and that PI3K activity was elevated in PC12 cells overexpressing rab3B. Furthermore, two structurally distinct inhibitors of PI3K (wortmannin and LY294002) inhibited NE uptake in intact as well as digitonin-permeabilized PC12 cells, but had no effect on calcium-evoked NE secretion. Our results indicate that rab3 and PI3K positively and coordinately regulate NE uptake in PC12 neuroendocrine cells at least in part by stimulating the secretory vesicle uptake step.

Transport and storage of the classical monoamines by neuroendocrine cells occur through the activities of two pharmacologically and functionally distinct transporters. Neurotransmitter uptake across the plasma membrane is facilitated by specific plasma membrane transporters that remove neurotransmitter from the extracellular milieu (1, 2). Vesicular monoamine transporters (VMATs)1 localized on the membranes of secretory vesicles concentrate monoamine neurotransmitters into large dense core vesicles (LDCVs), chromaffin granules, and secretory vesicles. VMAT is an electrogenic antiporter that uses a H+ electrochemical gradient maintained by an ATP-dependent vacuolar H+ pump located in the secretory vesicle membrane to drive neurotransmitter uptake. A number of drugs, including reserpine and tetrabenazine, inhibit neurotransmitter uptake by VMAT (3, 4). In both neurons and neuroendocrine cells, catecholamine transport across the vesicular membrane serves to rapidly and efficiently package newly synthesized and recycled catecholamines into secretory vesicles to control the supply of neurotransmitter available for exocytosis. Catecholamine uptake by neurons also helps terminate synaptic transmission by the removal of neurotransmitter from the synaptic cleft.

Both steps in catecholamine uptake (plasma membrane and vesicular membrane) are subject to regulation by second messengers that are in turn activated by receptor binding or membrane depolarization (5, 6). Neurotransmitter uptake is also regulated by heterotrimeric and monomeric GTPases. The heterotrimeric G-protein, Go2, inhibits catecholamine uptake by a neuroendocrine cell line (PC12) that accumulates norepinephrine into secretory granules by mechanisms similar to those utilized by chromaffin cells and noradrenergic neurons (7). In addition, monomeric GTPases of the rab3 subfamily can stimulate NE uptake by PC12 cells (8). rab3 GTPases are expressed in a variety of secretory cells including PC12 cells, where they localize to catecholamine-containing secretory granules (LDCVs). rab3 GTPases have been implicated in the regulation of exocytosis (8, 11, 12). Interestingly, however, we observed in a previous study that stably transfected PC12 cell lines that overproduce rab3A or rab3B also accumulated substantially greater amounts of exogenous NE than mock transfected or untransfected cells (8). This effect was greater for rab3B than rab3A and was seen for multiple rab3-overproducing clones (i.e. was not the result of clonal variation). This raises the interesting possibility that rab3 GTPases regulate not only the efficiency of secretion but also the amount of vesicle cargo available for secretion. The mechanism for the rab3-induced increased in NE uptake was not addressed in the earlier study and is the subject of the present investigation.

Here we addressed three outstanding questions concerning the regulation of NE uptake by rab3 GTPases in PC12 cells: (i) how does rab3B affect the kinetics (time course and concentration dependence) of NE uptake by intact PC12 cells, (ii) do rab3B and rab3A regulate NE uptake at the plasma membrane or the secretory granule membrane, and (iii) do rab3 GTPases interact with other molecules to regulate this process? We report that rab3B stimulates the rate and maximum accumulation of NE by PC12 cells, and that one of the sites of action for rab3B and rab3A is the secretory granule uptake step. Furthermore, we identify phosphoinositide 3-kinase (PI3K) as a putative rab3B effector protein and demonstrate that PI3K, like rab3B, positively regulates vesicular catecholamine uptake by

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1 The abbreviations used are: VMAT, vesicular monoamine transporter; LDCV, large dense core vesicle; NE, norepinephrine; GTP-γS, guanosine 5’-3-O-(thio)triphosphate; PI3K, phosphoinositide 3-kinase.

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PC12 cells. The involvement of PI3K in the regulation of NE uptake could be generally significant, because PI3K participates in a variety of cell signaling pathways that are activated by multiple growth factors and hormones (13, 14).

MATERIALS AND METHODS

Cell Culture—PC12 cells stably transfected with rab3A, rab3B, the GTP binding mutant rab3BN135I, and vector alone (mock) were generated as described (8). In our previous analysis of these clones, we observed that, like the endogenous rab3C in PC12 cells, recombinant rab3B and recombinant rab3B localized to LDCVs in the transfected cells (8).

PC12 cells were cultured in HEPES-HEPES RPMI with sodium bicarbonate and supplemented with 10% heat-inactivated horse serum, 5% fetal bovine serum, 1% penicillin/streptomycin (Sigma), and 400 units/ml hygromycin B (Calbiochem, La Jolla, CA) at 37 °C in a humidified incubator with 95% CO2 and 5% O2. All experiments were performed using PC12 cells cultured in the absence of nerve growth factor.

Production of Glutathione S-Transferase (GST) Fusion Proteins, in Vitro Binding Assays, and Immunoblotting—GST fusion proteins containing full-length human rab3B, rab3B, and the rat p58A regulatory subunit of PI3K were expressed in Escherichia coli using the pGEX-2T prokaryotic expression vector (Amersham Biosciences, Inc.). Transformed E. coli were induced with 100 μM isopropyl-1-thio-β-D-galactopyranoside at 37 °C for 3 h. GST fusion proteins were affinity purified from bacterial cell lysates with glutathione-agarose, as described (15).

The GST moiety was removed from GST-p85 PI3K by incubation with thrombin (1% thrombin w/v GST fusion protein) at 25 °C for 1 h. Thrombin was neutralized by incubation with p-aminobenzamidine (Sigma) for 1 h at 4 °C. 1 μg of immobilized GST, GST-rab3B, or GST-rabB5 prebound to 0.5 ml GDP or GTPγS was mixed with 1 μg of p85 GST moiety removed in the following buffer (20 mM Tris-HCl, pH 8.0, 100 mM NaCl, 10 mM MgCl2, 10% glycerol, and 1 mM dithiothreitol) supplemented with leupeptin, aprotonin, and pepstatin A (10 μg/ml) and 2 mM phenylmethylsulfonyl fluoride for 1 h at 4 °C. After 1 h, the bound proteins were washed extensively with the same buffer, and then eluted in 5× SDS sample buffer. The samples were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), transferred to polyvinylidene difluoride membrane (Bio-Rad), and blotted with a monoclonal antibody to p58A (Transduction Laboratories, Lexington, KY).

For Western blot analysis, PC12 cells were grown on 10-cm poly-1-lysine-coated dishes, washed in phosphate-buffered saline and lysed in 1% Nonidet P-40, 150 mM NaCl, 20 mM HEPES (pH 7.4), 1 mM EDTA, 10 μg/ml leupeptin, aprotonin, and pepstatin A, and 2 mM phenylmethysulfonyl fluoride for 14,000 g (14,000 × g) for 15 min. Protein concentrations of cell lysates were determined using the Micro BCA protein assay reagent kit (Pierce), and the amount of total protein (mg) in the lysate. $K_m$ and $V_{max}$ values were determined from plots of normalized transport activity versus total NE concentration (see “Results”).

Measurement of Vesicular NE Uptake in Digitonin-permeabilized Cells—NE uptake by digitonin-permeabilized cells was performed as described (16). Whole cells were incubated on 12-well poly-l-lysine-coated dishes, cultured for 2 days, and then permeabilized with 0.1 μM digitonin in complete media for 15 min at room temperature. The cells were then rinsed in HTMS buffer containing 20 mM HEPES, 20 mM Tris-HCl, 6 mM MgCl2, 0.3 mM sucrose (pH 7.4). To initiate uptake assays, the cells were incubated 30 min at room temperature with HTMS buffer containing 4 mM ATP and 4 μCi of [3H]NE in the presence of 1 mM glucose and 0.5% Tween 20 (pH 7.0). Included were the indicated inhibitors. Uptake was terminated by removal of [3H]NE-containing buffer. Cells were rinsed, chased at room temperature for 30 min in HTMS buffer without [3H]NE, and then lysed in 0.25 N NaOH. The amount of [3H]NE in the lystate was determined by liquid scintillation counting.

Sucrose Gradient Fractionation of [3H]NE-labeled PC12 Cells—In tact PC12 cells were loaded with [3H]NE for 1 h at 37 °C as described above. The cells were then homogenized in 10 mM HEPES, 0.32 mM sucrose buffer (1 × 40 strokes with a Potter homogenizer) and centrifuged at 14,000 rpm to pellet unbroken cells. The clarified homogenate was layered on a 0.6–2.2 M sucrose gradient with a 2.2 M sucrose pad (10-ml volume). The gradient was centrifuged at 110,000 g for 3 h at 4 °C with ultracentrifugation at 4,000 rpm. An equal aliquot of each fraction was analyzed by liquid scintillation counting for [3H]NE and by Western blotting for markers of LDCVs (secretogranins) and light vesicles (synaptophysin).

Measurement of PI3K Activity in Immunoprecipitates—PC12 cells plated on 10-cm poly-1-lysine-coated dishes were cultured until confluent. Cells were lysed in lysis buffer containing 20 mM Tris, pH 7.5, 1% Nonidet P-40, 157 mM NaCl, 135 mM KCl, 5 mM MgCl2, 5 mM CaCl2, 4 mM Na3VO4, and 10% glycerol supplemented with leupeptin, aprotonin, and pepstatin A (all at 10 μg/ml) and 2 mM phenylmethylsulfonyl fluoride for 5 min at 4 °C. Lysates were clarified by centrifugation at 14,000 g. 10 μg of anti-phosphotyrosine (PY20) (Transduction Laboratories), anti-p85 PI3K (Transduction Laboratories), or normal mouse IgG (Santa Cruz Biotechnology Inc., Santa Cruz, CA) were mixed for 2 h at 4 °C with 40 μg of protein A-Sepharose that were preadsorbed with mouse IgG (Santa Cruz Biotechnology Inc.). After washing extensively with the same buffer, a monoclonal antibody to p58A (Transduction Laboratories) and 2 μg of the rabbit polyclonal antibody to p85A (Transduction Laboratories) were added to the lysates followed by mixing for an additional 2 h. To determine whether rab3B interacts with PI3K, 25 μg of anti-rab3B polyclonal IgG (8, 16) bound to cyanoagen bromide-Sepharose was used for the immunoprecipitation step.

Immunoprecipitates were washed extensively first in lysis buffer and then in kinase assay buffer (20 mM β-glycerophosphate, 0.8 mM Na3VO4, 5 mM sodium pyrophosphate, 30 mM NaCl, 5 mM MgCl2). The lipid kinase reaction was performed at 30 °C for 10 min in a 70-μl kinase assay buffer containing 60 μg/ml phosphatidylinositol (Avanti Polar Lipids, Alabaster, AL), 3 μM cold ATP, and 30 μCi of [γ-32P]ATP (DuPont-NEN). Reactions were terminated with methanol:chloroform:methanol (20:10:1) and then separated on polytacisoxolate-coated thin layer chromatography (TLC) plates in methanol:chloroform:ammonium hydroxide:water (100:30:15:5.27). The phosphoinositide 3-phosphate lipid product was quantified by phosphorimaging analysis.

RESULTS

Previously we observed that recombinant rab3A and rab3B localized to LDCVs in PC12 cells and markedly stimulated the steady-state accumulation of radiolabeled NE by these cells (8). We observed no discernible differences in either the total number of LDCVs per cell or the number of LDCVs within 110 nm of the plasma membrane (8), suggesting that other factors were responsible for the observed effects of rab3 on NE uptake. In the present study we addressed the mechanism by which rab3 and other GTPase regulate catecholamine uptake by PC12 neuroendocrine cells. We focused on rab3A because this GTPase had quantitatively greater effects on NE uptake, although rab3A appears to exhibit qualitatively similar effects (Ref. 8; see below).

rab3B Stimulates the Rate and Maximal Accumulation of NE by Intact PC12 Cells—Fig. 1 shows the effects of rab3B on NE uptake in PC12 cells. The involvement of PI3K in the regulation of NE uptake could be generally significant, because PI3K participates in a variety of cell signaling pathways that are activated by multiple growth factors and hormones (13, 14).
rab3B increases the rate of NE uptake and its maximal accumulation by intact PC12 cells. A, time course of NE uptake by rab3B-expressing PC12 cells and mock transfected cells. Data are plotted as the amount of [3H]NE accumulated in equal numbers of cells expressed as counts/min (cpm) versus the time of incubation. B, maximal accumulation of radiolabeled NE is enhanced in rab3B-expressing cells. Cells were exposed to a constant concentration of [3H]NE (343 nM) and varying concentrations of unlabeled NE for 30 min. Plotted is the uptake of total NE (radiolabeled plus unlabeled) as a function of total NE concentration. Data are expressed as the mean ± S.D. Experiments were performed in duplicate three times with similar results. The data shown in Fig. 1B was fit to single Michaelis-Menten functions with the following parameters: rab3B: V_{max} = 343.6 ± 10.3 fmol/min mg; K_m = 1.82 ± 0.11 μM; R^2 = 0.999; mock: V_{max} = 91.0 ± 12.9 fmol/min mg; K_m = 1.84 ± 0.55 μM; R^2 = 0.967.

Fig. 2. rab3B increases the accumulation of exogenous NE into secretory granules. A mock and a rab3B PC12 clone were allowed to accumulate [3H]NE for 60 min. Membranes were fractionated as described under “Materials and Methods.” An equal aliquot of each fraction from each sample was analyzed by liquid scintillation counting. Fractions enriched in secretory granules and light vesicles were identified by immunoblotting with antibodies against secretogranin and synaptophysin, respectively.
rab3 and PI3K Stimulate Catecholamine Uptake

rab3 and PI3K Stimulate Catecholamine Uptake

rab3 Interacts Physically and Functionally with PI3K—To further characterize the mechanism by which rab3B regulates NE transport, we sought to identify putative rab3B effectors that might function in concert with rab3B to stimulate NE uptake. PI3K attracted our attention because of its role in vesicular trafficking and signal transduction; processes in which rab proteins and monomeric GTPases in general are thought to play important roles. In addition, we reported previously that rab3B expression in PC12 cells affects the profile of tyrosine phosphorylated proteins that co-immunoprecipitate with the 85-kDa regulatory subunit of PI3K (24). To determine whether there is an interaction between rab3B and PI3K, we immunoprecipitated rab3B from lysates of rab3B-expressing PC12 cells and tested for co-immunoprecipitated PI3K activity using an in vitro lipid kinase assay. Mock-transfected cells lacking detectable rab3B (8) served as negative controls. A rab3B-specific antibody (8, 16) precipitated 4-fold more PI3K activity from rab3B-expressing PC12 cells as compared with the mock transfected cells (Fig. 4A). To test whether native rab3B can also interact with PI3K in cells that normally express endogenous rab3B from HT29-CL19A colonic epithelial cells that express more endogenous rab3B than PC12 cells (8, 16) and observed 10–15 times more PI3K activity in these immunoprecipitates as compared with nonimmune controls (performed two times; results not shown). Unfortunately, our attempts to show an interaction between rab3A and PI3K using this strategy were unsuccessful because we could not immunoprecipitate rab3A from PC12 cells with any of three available antibodies.

To determine whether the association of rab3B with PI3K is mediated directly through one of the PI3K subunits or indirectly through an adapter molecule, we performed a direct pairwise binding assay with GST-rab3B and recombinant p85
rab3 and PI3K Stimulate Catecholamine Uptake

PI3K Regulates NE Uptake in Intact and Digitonin-permeabilized PC12 Cells—Because PI3K physically and functionally interacts with rab3B, we determined whether PI3K, like rab3B, can regulate NE uptake in PC12 cells. We used two structurally distinct inhibitors of PI3K activity, LY294002 and wortmannin, to test for an involvement of PI3K in the regulation of NE uptake. Each inhibitor produced a dose-dependent decrease in NE uptake in intact mock and rab3B-expressing cells (Fig. 6). The rab3B-expressing cells and the mocks exhibited similar decreases in NE uptake in response to either PI3K inhibitor. LY294002 at 50 μM also reduced NE uptake by the rab3A and rab3BN135I clones (results not shown). These findings indicate that PI3K activity, like rab3, positively regulates NE uptake in intact PC12 cells, and that PI3K modulates NE transport even in the absence of rab3A or rab3B overexpression (i.e. in the mocks).

We also examined the effects of LY294002 on NE uptake over a range of NE concentrations to determine how PI3K affects uptake kinetics (Fig. 7). Compared with vehicle-treated controls, LY294002-treated rab3B-expressing cells showed a 2-fold decrease in NE uptake compared with mock cells at all NE concentrations tested.
diminution in apparent $V_{\text{max}}$ as well as a significant decrease in the apparent affinity for NE (Fig. 7 legend). Thus, the effects of the PI3K inhibitor on uptake kinetics were somewhat more complicated than that of rab3B expression, which significantly complicated the uptake kinetics, we also counted the total numbers of LDCVs per cell and within 110 nm of the plasma membrane to determine whether this inhibitor had any effect on the number or distribution of LDCVs within PC12 cells. Table I shows that treatment with LY294002 for 6 h had no apparent effect on LDCV number or distribution in PC12 cells.

Finally, we determined whether PI3K, like rab3B, influences NE uptake at the vesicle membrane by testing the effects of PI3K inhibitors on NE uptake in digitonin-permeabilized cells. Fig. 8 shows that NE uptake in digitonin-permeabilized cells was inhibited in the presence of LY294002 or wortmannin during the 30 min uptake period. The results of the PI3K inhibitor experiments indicate that PI3K activity is a significant factor in the control of monoamine uptake at the granule surface.

**DISCUSSION**

In an earlier study we observed that rab3 isoforms stimulate the steady-state accumulation of exogenous NE by PC12 cells (8). The mechanism underlying the rab3-induced increase in NE uptake was not addressed in the previous study and was the impetus for the present investigation. Neurotransmitter uptake can be regulated at different levels; thus, a focus of this study was to examine the role of PI3K in the control of NE uptake. Our findings suggest that PI3K activity is a significant factor in the control of NE uptake in PC12 cells. Moreover, these results provide insights into the role of PI3K in the regulation of monoamine uptake and may have implications for understanding the mechanisms underlying neurotransmitter release and synaptic plasticity.
study was to identify the site of regulation by rab3 (i.e. plasma membrane or secretory granule). Because rab GTPases generally interact with accessory proteins to perform their function, an additional goal was to identify rab3 binding partners and to determine whether such binding partners also participate in the regulation of NE uptake.

The results of our kinetic analysis revealed that rab3B expression primarily increased the apparent $V_{max}$ of NE uptake by intact PC12 cells with no significant effect on the $K_m$. The kinetic data could be well fit by single Michaelis-Menten functions suggesting that one of the two uptake steps (plasma membrane or LDCV membrane) was limiting. Because rab3B and rab3A also increased NE accumulation in digitonin-permeabilized cells, the simplest explanation of our kinetic data is that monoamine uptake into secretory granules is the rate-limiting step. NE uptake by permeabilized cells was ATP-dependent, reserpine-sensitive and nomifensine-insensitive, as expected for VMAT-mediated uptake at the LDCV membrane. Thus, our data strongly indicate that these rab3 GTPases modulate NE uptake at least in part at the level of the secretory granule membrane.

The increase in apparent $V_{max}$ induced by rab3B expression could be explained in one of several ways: (i) increased numbers of secretory granules, (ii) increased numbers of NE transporters (VMAT) per granule, and (iii) increased proton motive driving force (voltage plus pH gradient) for NE uptake into secretory granules mediated by VMAT (7). Our morphologic results indicate that neither rab3B (8) nor PI3K (Table I) appear to affect the numbers or distributions of secretory granules in PC12 cells. At present we cannot distinguish between the latter two possibilities, at least with respect to the long term effects of rab3B expression on the uptake process (see below for discussion of the effects of the PI3K inhibitors on NE uptake).

An additional goal of this study was to identify putative rab3B effectors and to determine whether they participate in the regulation of NE uptake. Like other GTPases, rab proteins perform their functions through interactions with effector molecules. Using two different biochemical assays, we have identified PI3K as a rab3B-binding protein. Other monomeric GTPases, including Ras, Rac, and cdc42, have been shown to interact with PI3K (27–29). Ras interacts with the p110 catalytic subunit, and Rac and cdc42 can modulate PI3K activity by interacting with the p85 regulatory subunit. A possibility that is consistent with the data presented here would be that PI3K functions as an effector for rab3B. In accordance with criteria that classify proteins as rab effectors, the current data demonstrate that: (a) PI3K (in particular, the p85a regulatory subunit) binds preferentially to the active, GTP-bound conformation of rab3B; (b) rab3B modulates the lipid kinase activity of PI3K; and (c) PI3K activity regulates the same cellular process regulated by rab3B, namely NE uptake into LDCVs.

Several observations made in this and a previous study (24) are consistent with the proposal that rab3B controls PI3K activity by facilitating its interaction with tyrosine phosphoproteins and/or through direct binding to the p85 regulatory subunit. There is evidence that the basal function of p85 is to inhibit the activity of the p110 catalytic subunit and that binding of p85 to tyrosine phosphoproteins overcomes this inhibition (30). We observed increased PI3K activity in phosphotyrosine immunoprecipitates from rab3B- and rab3A-expressing cells. This elevation in activity was not because of an increase in tyrosine phosphorylation of the p85 subunit, implying that rab3B potentiates the recruitment of PI3K to tyrosine phosphoproteins. Consistent with this observation, we previously reported that rab3B expression affected the profile of tyrosine-phosphorylated proteins that co-immunoprecipitate with p85 (24). A role for rab3 in facilitating the interaction of p85 with tyrosine phosphoproteins would result in activation of PI3K activity. The elevated lipid kinase activity measured in p85 immunoprecipitates is also consistent with the proposal that PI3K is more active in cells expressing rab3B.

Given that rab3B both interacts with PI3K and stimulates NE uptake, we investigated whether PI3K could also participate in the regulation of NE uptake. Our results obtained with the PI3K inhibitors, LY294002 and wortmannin, implicate PI3K activity in the regulation of NE uptake. These inhibitors have no effect on the efficiency of NE secretion induced by raising cytosolic Ca$^{2+}$ (Ref. 24, and results not shown); thus, the involvement of PI3K in NE transport appears to be limited to NE uptake. Presumably rab3B utilizes other effectors to regulate secretion (e.g. see Refs. 31–33). Inhibiting PI3K had somewhat complicated effects on the kinetics of NE uptake involving changes in both the apparent $K_m$ and $V_{max}$. Perhaps this indicates that PI3K regulates NE uptake in intact cells at multiple levels (i.e. plasma membrane and LDCV membrane). NE uptake in mock-transfected cells was also inhibited by the PI3K inhibitors indicating that a basal level of PI3K activity, possibly involving endogenous rab3A, positively regulates NE uptake.

We found that PI3K activity regulates NE uptake at least in part at the level of the granule membrane. Both PI3K inhibitors caused a substantial reduction in NE uptake by digitonin-permeabilized cells over a relatively short period of time (30 min of incubation). The rapid effect of both PI3K inhibitors on NE uptake would seem to rule out an effect on the numbers of secretory granules (Table I) or the numbers of VMAT molecules. As discussed above, it is conceivable that PI3K and rab3B regulate vesicular NE uptake by affecting one or both of the driving forces for uptake across the granule membrane (i.e. the transmembrane pH gradient and the transmembrane voltage). Go$_{q10}$ inhibits NE uptake by PC12 cells by reducing the proton electrochemical gradient across the LDCV membrane (7). Future experiments will be required to determine whether rab3B and/or PI3K affect NE uptake across the LDCV membrane by this or other mechanisms.

In summary, we have shown that rab3B stimulates NE uptake at the level of the granule membrane; thus, this GTPase can regulate both limbs of NE transport (granule uptake (this study) and calcium-induced release (Ref. 8)). We identified PI3K as a rab3B binding partner, showed that PI3K activity is enhanced in cells expressing rab3, and observed that PI3K inhibitors reduced NE uptake in intact and permeabilized PC12 cells. Given that rab3B can interact with PI3K, it is reasonable to conclude that rab3B stimulates NE uptake, in part, through its interaction with PI3K. It is also possible that rab3B can affect uptake by mechanisms not involving PI3K, as suggested by the fact that NE uptake was still elevated in rab3B-expressing cells relative to mock cells, even in the presence of the highest concentrations of PI3K inhibitors that were tested (Fig. 7). As PI3K can participate in multiple signaling pathways, the results of this and future studies could provide novel insights into the regulation of NE uptake in neuroendocrine cells and perhaps neurons in various physiological settings.

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