Characterization of ATP Transport into Chromaffin Granule Ghosts

SYNERGY OF ATP AND SEROTONIN ACCUMULATION IN CHROMAFFIN GRANULE GHOSTS*

Laurie A. Bankston† and Guido Guidotti§
From the Department of Molecular and Cellular Biology, Harvard University, Cambridge, Massachusetts 02138

(Received for publication, March 12, 1996)

ATP is an excitatory neurotransmitter that is stored and cosecreted with catecholamines from cells of the adrenal medulla. While the transport of catecholamines into chromaffin granule ghosts has been extensively characterized, there is little information on the mechanism of ATP transport into these structures. Here we show that ATP transport is driven by the electrical component of the electrochemical proton gradient created by the chromaffin granule membrane H⁺-ATPase, and that the accumulated nucleotide is released from the vesicles by inhibition of the H⁺-ATPase. ATP is an excitatory neurotransmitter with the unique feature that its final degradation product extracellularly is an amine transporter inhibitor, reserpine, causes the depletion of stored ATP (9).

The chromaffin granule membrane contains a vacuolar-type (V-type) H⁺-ATPase, which generates an electrochemical proton gradient, acidifying the granule interior (10, 11). Both the chemical and electrical part of the proton gradient energize transport of catecholamines and serotonin (5-HT)estransformed into chromaffin granules and chromaffin granule ghosts (lysed chromaffin granules that lack intragranular proteins, ATP, and catecholamines) (12–16). ATP transport into chromaffin granules, on the other hand, requires only the positive inside membrane potential (17, 18).

Since intact granules contain large amounts of ATP, transport into granules cannot be distinguished from exchange with intragranular ATP. Therefore, we decided to use chromaffin granule ghosts to characterize the vectorial transport of ATP.

In this paper, we show that the vectorial transport of ATP into chromaffin granule ghosts results in the accumulation of ATP, and that the transport is not due to exchange with intravesicular ATP. Our results contradict an earlier report describing transport into chromaffin granule ghosts, in which it was concluded that chromaffin granule membranes do not contain a membrane potential-dependent ATP transporter (19). We present a biochemical characterization of this ATP transport and propose a mechanism for the transport of ATP into acidified storage vesicles. We also demonstrate that there is a synergy between the accumulation of ATP and that of 5-HT.

**EXPERIMENTAL PROCEDURES**

Materials—Bovine adrenal glands were obtained fresh from Arena Brothers’ Slaughterhouse in Hopkinton, MA. [3H]5-HT (15–30 Ci/mmol), [α-32P]ATP (30 Ci/mmol), [α-32P]GTP (800 Ci/mmol), [α-32P]UTP (800 Ci/mmol) and [α-32P]MeNH2 (40–60 mCi/mmol) were obtained from DuPont NEN. [14C]CJCN− (56 mCi/mmol) was from Amersham. Bafilomycin A1 was a generous gift from Dr. Hans-Peter Fiedler at the University of Tübingen. All other chemicals were from Sigma. Nitrocellulose filters were from Schleicher & Schuell.

Preparation of Chromaffin Granule Ghosts—Chromaffin granule ghosts were purified essentially as described by Phillips and Apps (20). Diced medullae from 10–20 bovine adrenal glands were minced thoroughly and collected in 1–2 ml of buffer (0.3 M sucrose, 10 mM HEPES, pH 7, 1 mM EDTA, 5 μg/ml leupeptin, and 2 μg/ml pepstatin)/g of tissue. Next, they were minced in a blender for 30 s and then homogenized in 3–5 strokes with a glass/Teflon homogenizer. The homogenate was spun at 3,000 rpm in a GSA rotor for 10 min at 4 °C, and the supernatant was then spun at 11,000 rpm in a GSA rotor for 20 min at 4 °C. Pellets were suspended in a small volume of buffer (0.3 M sucrose, 10 mM HEPES, pH 7, and 1 mM EDTA) and then lysed by diluting 50–100-fold in low ionic strength buffer (5 mM HEPES, 5 mM EDTA). After lysing the granules for 1 h on ice, sucrose was added to 0.3 M and membranes were collected (41,000 × g, 30 min). Membranes were suspended in 1–2 ml of buffer (0.3 M sucrose, 10 mM HEPES, pH 7, and 1 mM EDTA)/g of medullae. Five milliliters of membranes were loaded

† This work was supported in part by National Institutes of Health Grant HL08893 (to G. G.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ Supported in part by National Institutes of Health Training Grant ST32GM07998-14.

§ To whom correspondence should be addressed: Dept. of Molecular and Cellular Biology, 7 Divinity Ave., Cambridge, MA 02138. Tel.: 617-495-2301; Fax: 617-495-8308.
onto step gradients: 4.5 ml of 0.4 M sucrose, 10 mM HEPES, 1 mM EDTA on top of 2.5 ml of 0.4 M sucrose, 10 mM HEPES, 1 mM EDTA in D_2O, and spun in a Beckman SW 40 at 40,000 rpm for 30 min. Chromaffin membranes, marked by their pink color, have a high lipid to protein ratio and band at the sucrose/sucrose D_2O interface, while the brown mitochondrial membranes pellet. Ghosts were aliquoted, frozen in liquid N_2 at 5–10 mg/ml, and stored at –70 °C for up to 1 year without loss of activity.

The catecholamine content of the ghosts was determined and found to be 13–16 nmol/mg protein, indicating that greater than 99% of the catecholamine granule contents had been removed from the preparation (intact granules contain approximately 2200 nmol/mg of catecholamine; Ref. 21).

Transport Assays—Transport assays were all done in essentially the same way: 200 μg of membrane protein were mixed with ATP, MgCl_2 (or MgATP), an ATP regenerating system (100 μg/ml creatine kinase and 5 mM phosphocreatine), and in some cases with serotonin (5-HT) and 1–2 μCi of radioactive substrate ([α-32P]ATP, [3H]5-HT) in the buffers indicated. Reactions were done at 37 °C, and the reaction was stopped by transfer to an ice bath. 100 μl of reaction mix was diluted into 2 ml of ice-cold buffer, and chromaffin granule ghosts containing trapped substrate were collected on 0.45-μm nitrocellulose filters, using a 10-manifold vacuum filter apparatus. The filters were then washed with 3 × 2 ml of ice-cold buffer (300 mM sucrose and 10 mM HEPES), dried, immersed in scintillation fluid and counted for radioactivity. When tritiated substrate was used, filters sat in scintillant overnight prior to counting.

Measurement of ΔpH—[(14)C]MeNH_2 distribution across the chromaffin granule membrane, as described by Johnson and Scarpa (15), was used to determine the pH gradient. Chromaffin granule ghosts containing trapped MeNH_2 were collected on nitrocellulose filters as described above for the other transport assays. The pH gradient is given by the expressions

$$\Delta p\text{H} = \log \frac{[\text{MeNH}_2]_{\text{ext}}}{[\text{MeNH}_2]_{\text{int}}}$$  \hspace{1cm} (Eq. 1)

and

$$\text{mV of } \Delta p\text{H} = -\frac{2.3R T}{F} \Delta p\text{H}$$  \hspace{1cm} (Eq. 2)

Measurement of Δψ—[(14)C]SCN distribution across the chromaffin granule membrane, as described by Johnson and Scarpa (15), was used to determine the membrane potential. Chromaffin granule ghosts containing trapped [14C]SCN were collected on nitrocellulose filters as described above for the other transport assays.

$$\Delta \psi = \frac{2.3RT [\text{SCN}^-]_{\text{int}}}{F [\text{SCN}^-]_{\text{ext}}}$$  \hspace{1cm} (Eq. 3)

HPLC Assays—A Beckman HPLC was equipped with an anion exchange column. Samples were prepared for HPLC and eluted with a gradient of NH_4H_2PO_4 and KCl as described by Pogolotti and Santi (22). Eluted material was detected by absorbance at 259 nm.

RESULTS

Active ATP Transport into Chromaffin Granule Ghosts—Fig. 1 shows the time dependence of bafilomycin A_2-sensitive ATP transport into chromaffin granule ghosts (bafilomycin A_2 is a specific inhibitor of the chromaffin granule membrane, V-type, H^+-ATPase (23)) in a sucrose buffer containing 6 mM ATP and 1 mM MgCl_2. A total of approximately 6 nmol of ATP associated with 1 mg of the ghost protein under these conditions (Fig. 1A). Bafilomycin A_2 inhibits a fraction of the ATP that associates with the ghosts: 0.5 ± 0.2 nmol/mg at 5 min and 2.0 ± 0.4 nmol/mg at 40 min; bafilomycin A_1-sensitive transport is shown in Fig. 1B. Direct measurement of ATP accumulated within chromaffin granule ghosts (HPLC experiments described below) suggests that most of the bafilomycin A_2-insensitive association is due to the binding of ATP to ghost membranes and to nitrocellulose filters. Oligomycin, which inhibits the mitochondrial H^+-ATPase (24), has no effect on the association of ATP (data not shown); therefore, the observed transport is into chromaffin granule ghosts and not into contaminating mitochondria.

The concentration dependence of bafilomycin A_2-sensitive ATP transport at 10 min is shown in Fig. 2A. An Eadie-Hofstee plot of the data is shown in Fig. 2B: K_{m(app)} = 2.9 ± 1.1 μM and V_{max} = 1.2 ± 0.3 nmol/mg/10 min.

GTP and UTP Are Also Transported into Chromaffin Granule Ghosts—Fig. 3 shows that ATP, GTP, and UTP are all substrates for the chromaffin granule nucleotide transporter. This lack of specificity for nucleotides was reported previously for transport into intact chromaffin granules (18) and distinguishes the chromaffin granule system from the ATP/ADP exchanger of mitochondria, which is highly selective for ATP and ADP and will not transport other nucleotides (25).

Direct Measurement of Transported Nucleotide by HPLC Demonstrates That ATP and ADP Are Accumulated and That Transport Depends on the Electrical Component of the Electrochemical Proton Gradient—In order to demonstrate that the observed transport was due to the accumulation of ATP as opposed to exchange with residual ATP in the preparation, the

FIG. 1. Time course of ATP uptake into chromaffin granule ghosts. Chromaffin granule ghosts were incubated in buffer containing 300 mM sucrose, 10 mM HEPES, pH 7, 6 mM ATP, 1 mM MgCl_2, 5 mM creatine phosphate, and 100 μg/ml creatine kinase for 5–60 min at 37 °C, as described under “Experimental Procedures.” A, the data shown, expressed as nmol/mg, are the averages of four measurements ± S.E., no bafilomycin A_2; B, 10 μM bafilomycin A_2, B, the data shown are the difference ± 10 μM bafilomycin A_2, between the points shown in A.
amounts of ADP and ATP in chromaffin granule ghosts incubated under various conditions were analyzed by anion exchange HPLC (Table I). Both ADP and ATP accumulated within the ghosts in a bafilomycin A1-sensitive manner. Although only ATP was added, ADP was also found, presumably as a result of hydrolysis by the H⁺-ATPase and other ATPases in the preparation. The accumulation of ADP within the ghosts suggests that it is also a substrate of the ATP transporter.

Approximately 1.02 ± 0.25 nmol of ADP and ATP/mg of protein are present in the chromaffin granule ghost preparation, a small fraction of the 531 ± 66 nmol/mg of protein that are associated with intact chromaffin granules (21). As shown, the amount of ADP and ATP associated with the ghosts does not increase upon the addition of 6 mM ATP at 0 °C (1.10 ± 0.21 nmol/mg). However, upon incubation at 37 °C, 6.55 ± 1.02 nmol/mg of ADP and ATP associate with chromaffin granule ghosts. When the H⁺-ATPase is inhibited by bafilomycin A1, the amount of nucleotide associated with the ghosts is 2.14 ± 0.65 nmol/mg, indicating that the transport of approximately 4 nmol/mg of ADP and ATP depends on the activity of the H⁺-ATPase. Dissipation of the pH gradient by the addition of 20 mM NH₄Cl (in the presence of 20 mM NH₄Cl, ΔpH = 0, as determined by ¹⁴C-MeNH₂ uptake) does not inhibit the ATP transporter (7.69 ± 0.84 nmol/mg), whereas dissipation of the membrane potential by the addition of 20 mM KSCN completely...
blocks transport. The amount of ADP and ATP accumulated in the presence of 20 mM KSCN (1.79 ± 0.08 nmol/mg) is roughly equivalent to the amount of accumulation in the presence of bafilomycin A1 (2.19 ± 0.65 nmol/mg). The conclusion is that ATP transport is energized by the positive inside membrane potential generated by the V-type, H^+-ATPase.

In addition, the amount of ADP and ATP that accumulate increases to 9.21 ± 0.23 nmol/mg when serotonin (5-HT) is added to the reaction. This effect of 5-HT on ATP accumulation is discussed in detail below.

Chloride Stimulates ATP Transport—It has been shown (15), and we have confirmed (Fig. 4A) that chloride increases the pH gradient and decreases the membrane potential of chromaffin granule ghosts, suggesting that the anion readily enters the ghosts. Since chloride lowers the membrane potential, we assumed that the presence of chloride would decrease ATP uptake. Surprisingly, as shown in Fig. 4B, chloride stimulates the transport of ATP (3.01 ± 0.29 nmol/mg/40 min at 50 mM KCl versus 1.07 ± 0.02 nmol/mg/40 min in sucrose buffer lacking chloride). This effect of chloride is less at 100 mM KCl (1.81 ± 0.38 nmol/mg/40 min), and 180 mM KCl inhibits ATP transport (0.05 ± 0.29 nmol/mg/40 min). In contrast, 5-HT transport is only marginally stimulated (4.26 ± 0.29 nmol/mg/40 min in 50 mM KCl versus 3.72 ± 0.24 nmol/mg/40 min in sucrose buffer lacking chloride) by increasing concentrations of chloride (Fig. 4B), while 180 mM KCl has an inhibitory effect (2.47 ± 0.20 nmol/mg/40 min of 5-HT at 180 mM KCl versus 3.72 ± 0.24 nmol/mg/40 min in sucrose buffer lacking chloride). The inhibitory effect of 180 mM KCl is presumably due to a reduction in the membrane potential. Evidently, at the high KCl concentration ATP transport that is energized only by the membrane potential is completely inhibited, whereas 5-HT transport that is energized by both the membrane potential and the pH gradient is only partially inhibited. In order to verify that this stimulation of ATP transport was a specific effect of chloride, we tested the effect of 20 mM potassium gluconate and found that it did not stimulate ATP transport (0.67 ± 0.04 to 0.15 ± 0.04 nmol/mg/40 min at 20 mM gluconate versus 1.07 ± 0.21 nmol/mg/40 min in sucrose buffer). This stimulation by chloride is either due to its ability to stimulate the chromaffin granule membrane H^+-ATPase (Moriyama and Nelson (26) have shown that the purified, V-type, H^+-ATPase from chromaffin granule membranes is stimulated by chloride) or to a direct effect on the nucleotide transporter itself.

ATP Associated with Granule Ghosts Is Releasable—Table II shows that most (79%) of the 4.23 nmol/mg of ATP transported into ghosts in 30 min is released when the ghosts are diluted 10-fold in buffer containing 10 μM bafilomycin A1 to inhibit the V-type, H^+-ATPase. Most (85%) of the accumulated serotonin (5-HT) is also released from ghosts under the same conditions. Approximately 50% of the accumulated ATP (2.27 nmol/mg) and 20% of the accumulated 5-HT (2.40 nmol/mg) are released from the ghosts upon dilution alone. The fact that the transported solutes can be released from the granule ghosts suggests that their uptake is energized by the electrochemical proton gradient and is not due to exchange with intravesicular substrates.

DIDS Inhibition of ATP Transport—Since DIDS is an inhibitor of anion transporters such as Band 3 (27, 28), we looked at the ability of DIDS to inhibit ATP transport into chromaffin granule ghosts. Fig. 5 shows the dose response of ATP transport activity to DIDS (K_i = 27.3 ± 9.6 μM). Since DIDS does not significantly inhibit the formation of the pH gradient (ΔpH = 0.89 in the presence of 20 μM DIDS and ΔpH = 0.96 in the absence of DIDS; DIDS inhibits ATP transport approximately 50% at this concentration), we conclude that it inhibits ATP transport preferentially as compared to the H^+-ATPase.

Atractyloside Inhibition of ATP Transport—It has been reported that atractyloside, an inhibitor of the mitochondrial ATP/ADP exchanger (25), inhibits nucleotide uptake by intact granules (17). We find that the effect of atractyloside depends on the relative concentrations of ATP and Mg^2+. As is shown in Fig. 6, when most of the ATP is complexed with Mg^2+, 2 mM ATP and 2 mM Mg^2+ or 6 mM ATP and 6 mM Mg^2+, transport is inhibited approximately 50% by 200 μM atractyloside (0.3 ± 0.04 to 0.15 ± 0.07 nmol/mg/40 min for 2 mM ATP and 1.1 ± 0.2 to 0.5 ± 0.1 nmol/mg/40 min for 6 mM ATP). On the other hand, transport is not inhibited by 200 μM atractyloside (0.3 ± 0.04 to 0.15 ± 0.07 nmol/mg/40 min for 2 mM ATP and 1.1 ± 0.2 to 0.5 ± 0.1 nmol/mg/40 min for 6 mM ATP). On the other hand,
with both 2 mM and 6 mM ATP and 1 mM Mg²⁺ (0.43 ± 0.08 nmol/mg/40 min for 2 mM ATP and 2.18 ± 0.18 nmol/mg/40 min for 6 mM ATP), there is no effect of atractyloside on ATP transport. Interestingly, the amount of ATP transported at a given concentration of ATP is greater when the Mg²⁺ concentration is 1 mM rather than equal to the concentration of ATP. Synergy of ATP and Serotonin Transport into Chromaffin Granule Ghosts—Since the non-ideal behavior of solutions of catecholamines and ATP suggests that they interact (8), we wondered whether the accumulation of serotonin (5-HT) and ATP might be coupled, such that larger amounts of both solutes accumulate when they are taken up together. Fig. 7A shows that the uptake of ATP at 6 mM ATP increases approximately 2-fold as the concentration of 5-HT increases from 0 to 400 μM; under the same conditions, the uptake of ATP increases from 0 to 25 nmol/mg/40 min (Fig. 7B). Thus at 50 μM 5-HT, 3.0 ± 0.1 nmol/mg/40 min of ATP and 8.2 ± 0.3 nmol/mg/40 min of 5-HT are transported, while at 400 μM 5-HT, 5.9 ± 0.6 nmol/mg/40 min of ATP and 24.8 ± 0.8 nmol/mg/40 min of 5-HT are trans-located (data not shown). The evidence is expressed as nmol/mg/40 min, are the average of four measurements ± S.E. The amount associated with ghosts in the presence of 10 μM bafilomycin A₁, determined as the average of four measurements ± S.E., was subtracted from each value shown.

with both 2 mM and 6 mM ATP in buffer containing 300 mM sucrose, 20 mM KCl, 10 mM HEPES, pH 7, 1 mM MgCl₂, 5 mM creatine phosphate, and 100 μg/ml creatine kinase for 30 min at 37°C. To test for the reversibility of transport, samples were then diluted 1:10 in sucrose buffer with or without 10 mM bafilomycin A₁, and incubated an additional 30 min at 37°C. The data shown are the averages of four measurements ± S.E. The amount associated in the presence of 10 mM bafilomycin A₁, determined as the average of four measurements ± S.E., has been subtracted from each value shown.

**TABLE II**

| Substrate | 30 min | 30 min + 30 min at 1:10 dilution | 30 min + 30 min at 1:10 dilution + 10 mM bafilomycin A₁ |
|-----------|--------|-------------------------------|-------------------------------------------------------|
| ATP       | 4.23 ± 0.34 | 1.96 ± 0.27 | 0.04 ± 0.31 |
| 5-HT      | 12.02 ± 0.91 | 9.62 ± 0.97 | 2.02 ± 0.55 |

**FIG. 5.** DIDS inhibition of ATP transport. Chromaffin granule ghosts were incubated with 200 μM 5-HT and 6 mM ATP in buffer containing 300 mM sucrose, 20 mM KCl, 10 mM HEPES, pH 7, 1 mM MgCl₂, 5 mM creatine phosphate, and 100 μg/ml creatine kinase for 30 min at 37°C, as described under “Experimental Procedures.” The data shown, expressed as nmol/mg/10 min, are the average of four measurements ± S.E. The amount associated in the presence of 10 μM bafilomycin A₁, determined as the average of four measurements ± S.E., was subtracted from each value shown.

**DISCUSSION**

We show here that: 1) ATP is transported into chromaffin granule ghosts (Kₘ = 2.9 mM and Vₘₐₓ = 1.2 nmol/mg/10 min, measured at 2 mM chloride) (Figs. 1 and 2); 2) this transport is due to the accumulation of ADP and ATP and is not due to exchange with intravesicular nucleotide (Table I); 3) the transport requires the membrane potential but not the proton gradient set up by the proton pump (Table I); 4) the trapped nucleotides can be released (Table I); 5) GTP and UTP are also substrates for the transporter (Fig. 3); 6) chloride stimulates ATP transport (Fig. 4); 7) DIDS (Kᵢ = 26 μM) inhibits this ATP transporter (Fig. 5), while atractyloside is a weak inhibitor (Fig. 6); 8) although transport requires magnesium, magnesium concentrations that are equal to or greater than the concentration of ATP inhibit ATP transport (Fig. 6); and 9) the amount of ATP transported can be substantial (6 nmol/mg), up to 38% of the amount of accumulated 5-HT (Fig. 7). These
features are considerably different from those of the ATP transport systems in yeast endoplasmic reticulum vesicles (29) ($K_m = 10 \mu M$ and $V_{max} = 1.2 \text{ nmol/mg/min}$), rat liver endoplasmic reticulum vesicles (30) ($K_m = 4 \mu M$ and $V_{max} = 6.6 \text{ pmol/mg/min}$) and rat liver Golgi vesicles (31) ($K_m = 0.9 \mu M$ and $V_{max} = 58 \text{ pmol/mg/min}$).

Saturable uptake of nucleotides into intact chromaffin granules has been reported previously (17, 18, 32). However, this work has been brought into question for two reasons. First, intact granules contain large amounts of ATP; therefore, transport into granules cannot be distinguished from exchange with intragranular ATP. Second, the only report describing ATP transport into chromaffin granule ghosts (which do not contain appreciable amounts of ATP) concluded that chromaffin granule membranes do not contain an ATP transporter and that ATP enters chromaffin granules and ghosts by passive diffusion (19). Our clear demonstration of vectorial transport of ATP into chromaffin granule ghosts, resulting in the accumulation of nucleotide, refutes the latter argument. The common characteristics of ATP uptake into chromaffin granules and transport into ghosts are: 1) ATP, GTP, and UTP are all substrates; 2) transport is inhibited by proton ionophores and SCN$^-$ and not by ammonium ion; and 3) transport is inhibited by DIDS.

Table III. Synergy of ATP and 5-HT uptake

| Additions (substrate) | 2 mM ATP | 2 mM Mg$^{2+}$ | 6 mM ATP | 2 mM Mg$^{2+}$ |
|-----------------------|----------|----------------|----------|----------------|
| None                  | 0.16 ± 0.06 | 2.39 ± 0.28 | 0.18 ± 0.06 | 2.07 ± 0.32 |
| (ATP)                 | 0.48 ± 0.18 | 4.80 ± 0.53 | 0.53 ± 0.26 | 4.57 ± 0.63 |
| + 200 μM 5-HT (ATP)   | 10.02 ± 1.17 | 20.76 ± 1.43 | 11.76 ± 1.23 | 22.07 ± 1.34 |
| (5-HT)                |           |               |           |               |

Fig. 7. Effect of 5-HT transport on ATP transport into chromaffin granule ghosts. Chromaffin granule ghosts were incubated with increasing concentrations of 5-HT in buffer containing 300 mM sucrose, 20 mM KCl, 10 mM HEPES, pH 7, 2 mM ATP, 1 mM MgCl$_2$, 5 mM creatine phosphate, and 100 μg/ml creatine kinase for 40 min at 37°C. The data, ATP transport (A) and 5-HT transport (B), shown, expressed as nmol/mg/40 min, are the average of four measurements ± S.E. The amount associated in ghosts in the presence of 10 μM bafilomycin A$_2$, determined as the average of four measurements ± S.E., has been subtracted from each value shown.

Although transport of ATP into chromaffin granule ghosts can be detected in the absence of chloride, and high concentrations of chloride inhibit ATP transport by lowering the membrane potential, chloride stimulates transport at concentrations up to 100 mM (Fig. 4B). One possibility is that chloride stimulates the activity of the V-type, H$^+$/ATPase (26). Alternatively, chloride may stimulate the ATP transporter directly.

ATP transport into chromaffin granule ghosts is similar to that seen for the other negatively charged neurotransmitter, L-glutamate ($K_m = 1.6$ mM and $V_{max} = 13$ nmol/mg/min) (33-37). Like ATP transport, L-glutamate transport into synaptic vesicles is energized by the membrane potential generated by the synaptic vesicle membrane, V-type, H$^+$/ATPase, and it is inhibited by DIDS. In addition, low concentrations of chloride stimulate L-glutamate transport, while high concentrations of chloride inhibit it. While this effect of chloride may be due to the stimulation of the V-type, H$^+$/ATPase (26), chloride does not have this dramatic effect on the transport of other neurotransmitters that are also coupled to the V-type, H$^+$/ATPases (see, for example, the effect of chloride on 5-HT transport into chromaffin granule ghosts; Fig. 4B). Therefore, we propose that the effects of chloride are direct effects on the transporters for ATP and L-glutamate.

Since the uptake measured in these experiments is inhibited by bafilomycin A$_2$ and not by oligomycin, we are confident that there is no contribution from the mitochondrial ATP/ADP exchanger. Furthermore, GTP and UTP are also transported into chromaffin granule ghosts; these nucleotides are not substrates for the mitochondrial exchanger (25). However, both transporters can be inhibited by atractyloside, although the chromaffin granule system is only very weakly inhibited.

Importantly, there is a synergy between ATP and serotonin (5-HT) accumulation in chromaffin granule ghosts (Fig. 7 and Table III). At a given [ATP], there is a proportional increase in ATP accumulation with increasing extravesicular [5-HT]; similarly, at one [5-HT], there is an increase in ATP accumulation with an increase in extravesicular [ATP]. Evidently, the amounts of 5-HT and ATP taken up by chromaffin granule ghosts, under the proper conditions, are balanced so that one molecule of ATP with 3–4 negative charges is capable of neutralizing the positive charge of 3–4 5-HT molecules. If ATP$^{4-}$ is the substrate of the transporter, a possibility compatible with the effect of Mg$^{2+}$ on the transport activity, at an intravesicular
pH of 5.5 the accumulated ATP will have three negative charges. This preferential neutralization of accumulated ATP by 5-HT as opposed to protons suggests that there is an interaction between ATP and 5-HT that reduces the effective concentration of the two within the ghosts. The idea that biogenic amines and ATP interact is further supported by the observation that the osmolarity of a solution of 0.6 M epinephrine and 0.15 M ATP is 250 mosm, one third the expected osmolarity (8).

Acknowledgment—We thank Robert C. Liddington for critical reading of the manuscript.

REFERENCES
1. Evans, R. J., Derkach, V., and Surprenant, A. (1992) Nature 357, 503–505
2. Edwards, F. A., Gibb, A. J., and Colquhoun, D. (1992) Nature 359, 144–147
3. Rainie, D. G., Grunze, H. C. R., McCarley, R. W., and Greene, R. W. (1994) Science 263, 689–692
4. Pölsner, A. M., and Trifaro, J. M. (eds) (1982) The Secretory Granule, Elsevier Biomedical Press, Amsterdam
5. Hillarp, N. A. (1958) Acta Physiol. Scand. 42, 321–332
6. Hillarp, N. A., and Thieme, G. (1959) Acta Physiol. Scand. 45, 328–338
7. Kirshner, N. (1962) J. Biol. Chem. 237, 2311–2317
8. Kopell, W. N., and Westhead, E. W. (1962) J. Biol. Chem. 257, 5707–5710
9. Caughey, B., and Kirshner, N. (1967) J. Neurochem. 49, 563–573
10. Cidon, S., and Nelson, N. (1986) J. Biol. Chem. 261, 9222–9227
11. Forgac, M. (1989) Physiol. Rev. 69, 765–796
12. Phillips, J. H. (1974) Biochem. J. 144, 311–318
13. Phillips, J. H. (1978) Biochem. J. 170, 673–679
14. Schuldiner, S., Fishkes, H., and Kanner, B. (1978) Proc. Natl. Acad. Sci. U. S. A. 75, 3713–3716
15. Johnson, R. G., and Scarpa, A. (1979) J. Biol. Chem. 254, 3750–3760
16. Johnson, R. G., Carty, S. E., and Scarpa, A. (1981) J. Biol. Chem. 256, 5773–5780
17. Aberer, W., Kastron, H., Huber, E., and Winkler, H. (1978) Biochem. J. 172, 353–360
18. Weber, A., and Winkler, H. (1981) Neuroscience 6, 2269–2276
19. Grüninger, H. A., Apps, D. K., and Phillips, J. H. (1983) Neuroscience 9, 917–924
20. Phillips, J. H., and Apps, D. K. (1980) Biochem. J. 192, 273–278
21. Sillero, M. A. G., Del Valle, M., Zaera, E., Michelena, P., Garcea, A. G., and Sillero, A. (1994) Biochimie (Paris) 76, 404–409
22. Pogokoto, A. L., and Santi, D. V. (1982) Anal. Biochem. 126, 335–345
23. Bowman, E. J., Siebers, A., and Altendorf, K. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 7972–7976
24. Fillingame, R. H. (1980) Annu. Rev. Biochem. 49, 1079–1113
25. Klingenberg, M. (1985) in The Enzymes of Biological Membranes (Martinosi, A. N., ed) Vol. 4, pp. 511–553, Plenum Press, New York
26. Moriyama, Y., and Nelson, N. (1987) J. Biol. Chem. 262, 9175–9180
27. Cabantchik, Z. I., and Rothstein, A. (1974) J. Membr. Biol. 15, 227–248
28. Passow, H. (1986) Rev. Physiol. Biochem. Pharmacol. 103, 61–203
29. Mayinger, P., and Meyer, D. I. (1993) EMBO J. 12, 659–666
30. Clairmont, C. A., DeMaio, A., and Hirschberg, C. B. (1992) J. Biol. Chem. 267, 3983–3990
31. Capasso, J. M., Keenan, T. W., Abejon, C., and Hirschberg, C. B. (1989) J. Biol. Chem. 264, 5233–5240
32. Weber, A., Westhead, E. W., and Winkler, H. (1983) Biochem. J. 210, 789–794
33. Naito, S., and Ueda, T. (1985) J. Neurochem. 44, 99–109
34. Maycox, P. R., Deckworth, T., Hell, J. W., and Jahn, R. (1988) J. Biol. Chem. 263, 15423–15428
35. Cidon, S., and Sihra, T. S. (1989) J. Biol. Chem. 264, 8281–8288
36. Shioi, J., Naito, S., and Ueda, T. (1989) Biochem. J. 258, 499–504
37. Shioi, J., and Ueda, T. (1990) Biochem. J. 267, 63–68