Electrogenic Epinephrine Transport in Chromaffin Granule Ghosts†

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ABSTRACT: An ATP-dependent proton pump drives epinephrine transport in chromaffin granule ghosts. When ghosts are suspended in a medium free of permeant anions, ATP addition leads to an increase in membrane potential (interior positive) and epinephrine uptake but not to a change in intravesicular pH. Since ATP does not affect the pH gradient, the energy for transport must be drawn from the membrane potential ($\Delta\psi$), and epinephrine uptake must result in a net efflux of positive charge. This can be achieved by an antiport (exchange diffusion) mechanism in which each catecholamine cation is taken up in exchange for more than one H^+. Measurements indicate that the stoichiometry is close to 2 H^+/epinephrine cation, so the equilibrium epinephrine gradient is theoretically $[E]_{in}/[E]_{out} = ([H^+]_{in}/[H^+]_{out})^2 e^{F\Delta\psi/(RT)}$. In deenergized ghosts, the epinephrine concentration gradient equals the $[H^+]$ gradient. This is consistent with a situation in which the H^+ concentration gradient is in equilibrium with the membrane potential as described by the Nernst equation. Then, in the equation above, the membrane potential term ($e^{F\Delta\psi/(RT)}$) will exactly cancel one power of the $[H^+]$ gradient, leaving $[E]_{in}/[E]_{out}$ equal to $[H^+]_{in}/[H^+]_{out}$.

The chemiosmotic hypothesis proposed by Mitchell (1961) has provided a rationalization for many active transport systems (Harold, 1976). Protons are pumped across the membrane to create a transmembrane gradient in proton electrochemical potential ($\Delta\mu_{H^+}$). Molecules can then be actively transported by coupling their movement to the flow of H^+ down its potential gradient.

The transport of catecholamines into chromaffin granules (the storage vesicles of the adrenal medulla) is driven by an inwardly directed proton-translocating adenosine triphosphatase (Njus & Radda, 1978). The manner in which catecholamine transport is coupled to the proton pump, however, has caused considerable confusion. If a univalent cation, such as a catecholamine, is exchanged for a single H^+, there will be no net charge transfer and the transport will depend on the proton chemical potential gradient (ΔpH)[1] but not on the electrical potential gradient ($\Delta\psi$). On the other hand, if the cation is exchanged for more than one H^+, the transport will respond to the membrane potential as well as to the pH gradient. We have argued that catecholamine transport can be driven by the membrane potential and therefore is electrogenic (Casey et al., 1977; Njus & Radda, 1978, 1979). Holz (1978) and Johnson & Scarpa (1979) have presented evidence supporting this hypothesis. Several other groups, however, have advocated an electroneutral mechanism, one depending only on the pH gradient across the membrane (Aberer et al., 1978; Phillips, 1978; Schuldiner et al., 1978; Ingebretsen & Flatmark, 1979).

We showed that there are conditions under which the proton pump drives uptake but does not change the pH gradient. This implies that the membrane potential must provide the driving force (Casey et al., 1977). Holz (1978) and Johnson & Scarpa (1979) subsequently showed that the catecholamine uptake correlates well with the membrane potential. These studies, however, were all performed on intact chromaffin granules. Because chromaffin granules contain a complex matrix (Winkler, 1976) which may bind catecholamines or exchange internal for external catecholamine, transport is most clearly observed in granules that have been lysed and resealed to form "ghosts". Most studies on ghosts, however, have shown only that catecholamine uptake occurs in response to pH gradients (Phillips, 1978; Schuldiner et al., 1978; Ingebretsen & Flatmark, 1979). Although these investigators argue that transport is electroneutral, they have not tested the effect of the membrane potential itself on catecholamine transport.

We have recently shown that K^+ diffusion potentials, generated by using the ionophore valinomycin, cause epinephrine uptake into ghosts (Njus & Radda, 1979). The diffusion potential is transient, however, so only a short pulse of uptake is observed. Unambiguous evidence that catecholamine transport is electrogenic would be the demonstration in ghosts that ATP hydrolysis drives transport and generates a membrane potential but does not affect the pH gradient. We have developed a ghost preparation with a well-buffered internal space. In these ghosts, the proton pump generates a large $\Delta\psi$ but does not change the internal pH. This allows us to test the correlation between membrane potential and catecholamine transport in chromaffin granule ghosts.

Materials and Methods

Chromaffin granules were prepared from bovine adrenal glands (Njus et al., 1978). The granules were then lysed in 0.2 M Tris–phosphate, pH 7.0, and the ghost membranes were purified on Ficoll density gradients as described previously (Njus & Radda, 1979). All experiments were completed within 12 h of the cattle being slaughtered.

The pH gradient was calculated from the distribution of methylamine between the inside and outside of the ghosts (Schuldiner et al., 1972; Casey et al., 1977):

$$\Delta\text{pH} = \log \frac{[MeNH_3^+]_{in}}{[MeNH_3^+]_{out}} \quad (1)$$

Membrane potential was calculated from the thiocyanate distribution (Rottenberg, 1975; Drake et al., 1979) with the value of RT/F taken as 25.7 mV:

$$\Delta\psi = \frac{RT}{F} \ln \frac{[SCN^-]_{in}}{[SCN^-]_{out}} \quad (2)$$

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[1] Abbreviations used: ATPase, adenosine triphosphatase; FCCP, carbonyl cyanide *p*-(trifluoromethoxy)phenylhydrazone; Hepes, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; Mes, 2-(*N*-morpholino)ethanesulfonic acid; ΔpH, transmembrane pH gradient; $\Delta\psi$, transmembrane potential gradient; $[E]_{in}/[E]_{out}$, transmembrane epinephrine concentration gradient; V_{in}, internal volume.

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Table I: ΔpH, Δψ, and Epinephrine Uptake in KCl Medium[a]

additions	ΔpH	Δψ (mV)	$[E]_{in}/[E]_{out}$
none	0.55 ± 0.14	−1.1 ± 0.0	5.3 ± 0.8
ATP	0.92 ± 0.01	+17.2 ± 6.3	44.9 ± 3.3
FCCP	0.58 ± 0.05	+1.7 ± 5.7	3.5 ± 0.0
ATP + FCCP	0.41 ± 0.04	+2.8 ± 3.2	3.2 ± 0.2

[a] Ghosts (1.7 mg of protein in 0.25 mL) were added to 0.25 mL of 250 mM KCl, 150 mM sucrose, 40 mM Hepes, 2 mM $MgSO_4$, 200 μM sorbitol, 100 μM KSCN, and 100 μM methylamine, pH 7.0. 25 μL of 100 mM ATP, 100 mM $MgSO_4$, pH 7, 10 μL of 500 μM FCCP, and appropriate radioactive tracers were added to the samples as indicated. After incubation at 25 °C for 30 min, samples were centrifuged and processed as described under Materials and Methods. Values of ΔpH, Δψ, and $[E]_{in}/[E]_{out}$ are each the average of two replicate samples. These were calculated by using a sorbitol-excluding volume (V_{in} = 5.64 ± 0.92 μL) averaged from eight samples.

Table II: ΔpH, Δψ, and Epinephrine Uptake in Sucrose Medium[a]

additions	ΔpH	Δψ (mV)	$[E]_{in}/[E]_{out}$
none	0.79 ± 0.00	−9.8 ± 2.7	5.6 ± 0.3
ATP	0.68 ± 0.04	+46.0 ± 0.8	38.2 ± 0.9
FCCP	0.65 ± 0.15	−7.4	2.7 ± 2.1
ATP + FCCP	0.57 ± 0.03	+2.2 ± 0.7	3.2 ± 0.4

[a] Ghosts (2.4 mg of protein in 0.25 mL) were added to 0.25 mL of 0.4 M sucrose, 40 mM Hepes, 2 mM $MgSO_4$, 200 μM sorbitol, 100 μM KSCN, and 100 μM methylamine, pH 7.1. 25 μL of 100 mM ATP, 100 mM $MgSO_4$, pH 7, 10 μL of 500 μM FCCP, and appropriate radioactive tracers were added to the samples as indicated. After incubation at 25 °C for 30 min, samples were centrifuged and processed as described under Materials and Methods. Values of ΔpH, Δψ, and $[E]_{in}/[E]_{out}$ are each the average of two replicate samples. These were calculated by using a sorbitol-excluding volume (V_{in} = 4.0 ± 1.0 μL) averaged from eight samples.

The epinephrine concentration gradient is expressed as the ratio of the internal to external concentration ($[E]_{in}/[E]_{out}$). Internal and external concentrations of [^{14}C]methylamine, [^{14}C]thiocyanate, and [^{14}C]epinephrine were determined as previously described for $[MeNH_3^+]_{int}$ and $[MeNH_3^+]_{ext}$ (Casey et al., 1977). Ghosts were incubated as specified with 1 μCi of ^3H_2O and the appropriate ^{14}C-labeled compound (0.67 μCi of 50 mCi/mmol [^{14}C]methylamine, 0.72 μCi of 59 mCi/mmol potassium [^{14}C]thiocyanate, or 0.53 μCi of 50 mCi/mmol [^{14}C]epinephrine). After incubation for the specified time, samples were centrifuged at 25000*g* for 20 min at the incubation temperature. The pellets and supernatants were separated, processed, and counted as described (Casey et al., 1977). To calculate internal concentrations of methylamine, SCN^-, and epinephrine, it is necessary to know the volume V_{in} enclosed by the ghosts. This was measured as the [^{14}C]-sorbitol-excluding volume as described by Casey et al. (1977).

Protein was assayed by using biuret reagent as described by Casey et al. (1976). Error limits in all figures and tables are standard deviations calculated from multiple samples.

Carbonyl cyanide *p*-(trifluoromethoxy)phenylhydrazone (FCCP) was obtained from Sigma Chemical Co. [^{14}C]-DL-Adrenaline DL-bitartrate, potassium [^{14}C]thiocyanate, and [^{14}C]sorbitol were purchased from Amersham Corp., and ^3H_2O and [^{14}C]methylamine hydrochloride were from New England Nuclear.

Results

Radioisotope distribution techniques were used to measure the epinephrine concentration gradient ($[E]_{in}/[E]_{out}$), the membrane potential (Δψ), and the pH gradient (ΔpH) on the same preparation of ghosts under the same conditions. If chromaffin granules are suspended in a medium containing a permeant anion such as Cl^-, the anion will cross the membrane to dissipate the membrane potential. When ATP is added, H^+ will be translocated across the membrane and Cl^- will follow to neutralize the charge. In intact chromaffin granules, this H^+/anion influx causes a drop in intragranular pH (Casey et al., 1977). If Tris–P_i-loaded chromaffin granule ghosts are suspended in a KCl medium, the same effect can be seen (Table I). ATP causes a drop in intravesicular pH (an increase in ΔpH) and only a small increase in the membrane potential (Δψ). The ATP-dependent epinephrine gradient ($[E]_{in}/[E]_{out}$) is substantial. That the ATP-dependent changes are caused by proton translocation is indicated by the fact that they are reversed by the uncoupler FCCP.

In the absence of permeant anions (sucrose medium), proton translocation into intact chromaffin granules produces a membrane potential (interior positive) but does not change the intragranular pH (Casey et al., 1977). This is because the membrane potential created by H^+ translocation inhibits extensive proton pumping and the high internal buffering concentration precludes any noticeable change in internal pH. As seen in Table II, the same is true of Tris–P_i-loaded chromaffin granule ghosts. ATP addition increases the membrane potential but does not change ΔpH. This transfer of the H^+ potential energy gradient (proton motive force) from ΔpH to Δψ has only a small effect on ATP-dependent epinephrine uptake. Again, these ATP-dependent changes are inhibited by the uncoupler FCCP.

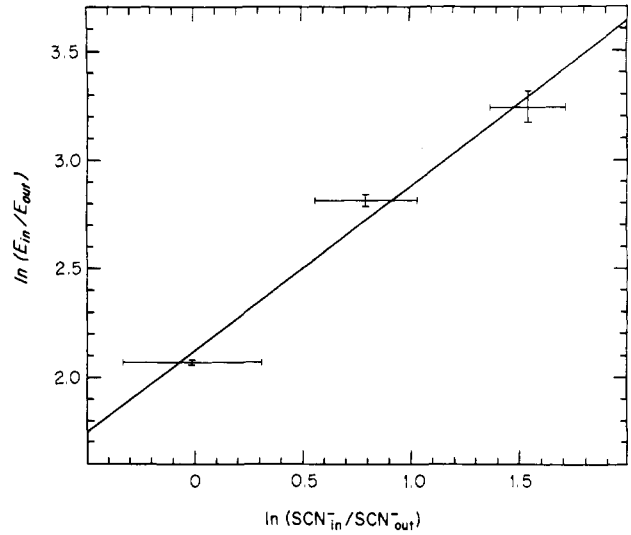

FIGURE 1: Relation between epinephrine gradient and thiocyanate distribution. $[E]_{in}/[E]_{out}$ and $[SCN^-]_{in}/[SCN^-]_{out}$ were determined in the presence of 0.5, 0.1, or 0 nmol of FCCP. Four replicate samples were averaged to determine each value. Values of ln ($[E]_{in}/[E]_{out}$) are plotted against values of ln ($[SCN^-]_{in}/[SCN^-]_{out}$) obtained at the same FCCP concentration. The straight line, representing a least-squares best fit to the data points, has a slope of 0.75 ± 0.10. For each sample, 0.25 mL of ghosts (2.0 mg of protein) was added to 0.25 mL of 0.4 M sucrose, 40 mM Hepes, 2 mM $MgSO_4$, 200 μM sorbitol, 100 μM KSCN, and 100 μM methylamine, pH 7.2. FCCP, 25 μL of 100 mM ATP, 50 mM $MgSO_4$, pH 7, and appropriate radioactive tracers were added. After incubation at 25 °C for 60 min, samples were centrifuged and processed as described under Materials and Methods. The average sorbitol-excluding volume (3.7 ± 1.8 μL) obtained from seven samples was used to calculate $[E]_{in}$ and $[SCN^-]_{in}$.

To observe the dependence of epinephrine uptake on the membrane potential, the latter was varied by adding different concentrations of the uncoupler FCCP. ln ($[SCN^-]_{in}/[SCN^-]_{out}$), which is equal to $[F/(RT)]\Delta\psi$ (eq 2), was used

Table III: Effect of Relysis on ΔpH and Δψ in Uncoupled Ghosts[a]

sample	ΔpH	Δψ (mV)	V_{in} (μL)
isotonic medium	0.87 ± 0.05	+6.9 ± 9.8	4.8 ± 0.1
hypotonic medium	0.60 ± 0.08	+15.2 ± 9.7	5.0 ± 1.3

[a] Ghosts (2.0 mg of protein in 0.25 mL) were added to 0.75 mL of buffer and 10 μL of 500 μM FCCP. Hypotonic buffer was 10 mM Hepes, 2 mM $MgSO_4$, and 200 μM sorbitol, pH 7.1. Isotonic buffer also contained 0.4 M sucrose. Appropriate radioactive tracers were added, and samples were centrifuged and processed as described under Materials and Methods. Values of ΔpH, Δψ, and V_{in} are each the average of two replicate samples. ΔpH and Δψ were calculated by using an internal volume of 4.9 μL.

as a measure of the membrane potential. The natural logarithms of the epinephrine gradient ($[E]_{in}/[E]_{out}$) and the SCN^- gradient are linearly related (Figure 1). The slope of this line determined in three separate experiments using different ghost preparations was 1.0 ± 0.2.

Chromaffin granule ghosts, lysed and resealed at pH 7, have a residual pH gradient of ~0.6 (Tables I and II), implying that the ghosts retain a large amount of trapped acid. Because the ghosts are loaded with 0.2 M Tris-P_i, they can be relysed by dilution into hypotonic medium. Relysing the ghosts causes the pH gradient to dissipate only slightly (Table III), indicating that the trapped acid must be rather tightly bound. Relysis also has little effect on the membrane potential.

In uncoupled ghosts, the pH gradient is nearly independent of the external pH (Figure 2). The epinephrine gradient closely parallels this H^+ gradient. The correlation between ΔpH [log ($[H^+]_{in}/[H^+]_{out}$)] and log ($[E]_{in}/[E]_{out}$) can also be seen in the uncoupled (FCCP) samples in Tables I and II. Values of log ($[E]_{in}/[E]_{out}$) for these FCCP samples are 0.54 (Table I) and 0.43 (Table II).

Discussion

The large pH gradients observed in resting ghosts (Tables I–III and Figure 2) have been reported before (Phillips & Allison, 1978; Drake et al., 1979). For several reasons, we believe that this gradient is real and not an artifact caused by methylamine binding to the ghosts. First, there is no evidence of methylamine binding to intact chromaffin granules. In intact granules, the pH determined from methylamine distribution (Johnson & Scarpa, 1976; Casey et al., 1977) agrees with the value obtained by using phosphorus-31 nuclear magnetic resonance (Njus et al., 1978; Pollard et al., 1979). Second, the epinephrine gradient parallels the methylamine gradient (Figure 2). This is reasonable if methylamine and epinephrine are both responding to the pH gradient. If methylamine and epinephrine were both binding to the ghosts, however, it is unlikely that their binding would be so similar. Consequently, we attribute this measured pH gradient to a Donnan equilibrium created by negatively charged groups sequestered within the ghosts.

Because our ghosts are prepared in nominally isotonic medium, a possible concern is that the ghost preparation is contaminated by unlysed chromaffin granules. The intact granules have a very low internal pH (~5.5), so a relatively small fraction (~20%) could account for the residual pH gradient. If this were the case, however, then diluting the granules into hypotonic medium should cause these granules to lyse and the measured ΔpH should disappear. Relysis has only a small effect on the measured internal pH (Table III), indicating that contamination by intact granules is not significant. The low catecholamine content of the ghost preparation (0.1 μmol/mg of protein compared to 1.5 μmol/mg in intact granules; D. Njus, unpublished observations) also argues against significant granule contamination.

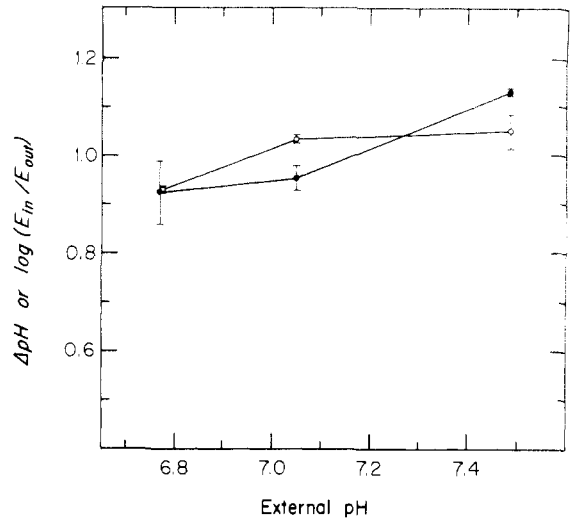

FIGURE 2: pH dependence of measured ΔpH and $[E]_{in}/[E]_{out}$. Each point is the average of two replicate samples. For each sample, 0.25 mL of ghosts (2.9 mg of protein) was added to 0.25 mL of 0.4 M sucrose, 40 mM Hepes, 2 mM $MgSO_4$, 200 μM sorbitol, 100 μM KSCN, and 100 μM methylamine at the pH indicated. 10 μL of FCCP and the appropriate radioactive tracers were added. After incubation at 26 °C for 30 min, samples were centrifuged and processed as described under Materials and Methods. The average sorbitol-excluding volume (3.9 ± 1.6 μL) obtained from six samples was used to calculate $[E]_{in}/[E]_{out}$ and ΔpH.

It has been known for many years that weak bases (EH_2^+) cross lipid membranes in their neutral form but not in their protonated form. For this reason, weak bases distribute across vesicle membranes according to the pH gradient (Waddell & Bates, 1969) and accumulate inside acidic vesicles:

$$\frac{[EH_2^+]_{in}}{[EH_2^+]_{out}} = \frac{[H^+]_{in}}{[H^+]_{out}} \quad (3)$$

Because intact chromaffin granules have an acidic internal pH, they take up high concentrations of the weak base methylamine, and it is tempting to think that catecholamines accumulate inside the granules by this sort of mechanism. In recent months, several groups have suggested this hypothesis (Phillips, 1978; Aberer et al., 1978; Schuldiner et al., 1978; Ingebretsen & Flatmark, 1979). We have argued before, however, that this mechanism is highly unlikely since ATP-dependent catecholamine uptake occurs in media which do not support an ATP-dependent change in intragranular pH (Casey et al., 1977; Njus & Radda, 1978).

We have recently developed a procedure for making ghosts that contain a high concentration of buffer. We have shown here that these ghosts, when suspended in a sucrose medium, maintain a stable internal pH in the face of ATP-dependent proton translocation (Table II). Under these conditions, ATP hydrolysis generates a membrane potential and increases epinephrine uptake over 10-fold although the pH gradient does not change. The electroneutral mechanism (eq 3) accounts for epinephrine gradients in uncoupled ghosts, but pH gradients are not nearly large enough to account for ATP-dependent epinephrine gradients (Table IV). In these experiments, uptake was measured after only 30 min and would not yet have reached equilibrium levels. Measured gradients, therefore, should be less than the calculated values. Since ΔpH does not account for ATP-dependent epinephrine uptake, the energy for transport must be drawn from the membrane potential. This, together with our previous demonstration of

Table IV: Comparison of Measured Epinephrine Gradients with Maximum Gradients Allowed by the Electroneutral and Electrogenic Mechanisms[a]

sample	ΔpH	Δψ (mV)	$[E]_{in}/[E]_{out}$ measured	$[E]_{in}/[E]_{out}$ electroneutral	$[E]_{in}/[E]_{out}$ electrogenic
active (ATP)					
KCl medium	0.92	+17.2	44.9	8.3	135
sucrose medium	0.68	+46.0	38.2	4.8	137
uncoupled (FCCP)					
KCl medium	0.58		3.5	3.8	
sucrose medium	0.65		2.7	4.5	

[a] Values of Δψ, ΔpH, and measured $[E]_{in}/[E]_{out}$ are taken from Tables I and II (ATP and FCCP samples). Values of Δψ for uncoupled samples are omitted because these are small potentials not accurately measured by thiocyanate distribution. The last two columns show values for $[E]_{in}/[E]_{out}$ calculated from Δψ and ΔpH by using eq 3 and 5, respectively. In calculations using eq 5, n was assigned a value of unity.

epinephrine uptake driven by K^+ diffusion potentials (Njus & Radda, 1979), provides strong evidence that epinephrine uptake is electrogenic.

Catecholamines are positively charged at neutral pH, yet uptake is driven by a membrane potential (interior positive). Consequently, uptake must be coupled to a cation (H^+) efflux or an anion (OH^-) influx. It is not possible at present to distinguish between H^+- and OH^--linked mechanisms, but the two are thermodynamically equivalent. Consequently, we will consider the catecholamine transport system as H^+ linked, remembering that a similar analysis would apply if the mechanism were OH^- linked.

Much has been said of the possibility that catecholamines behave as weak bases. It should be recognized, though, that catecholamines have two ionizable groups, the amino with a pK_a of ~9.9 and the catechol hydroxyls with a pK_a of about 8.8. At neutral pH, therefore, the predominant species is the cation. Roughly 2% is in the zwitterionic form, 0.2% is in the neutral form, and just a trace is in the anionic form. We do not know which of these species is actually transported across the membrane. Johnson & Scarpa (1979) have suggested that the neutral molecule is transported, but we do not feel that the evidence for this is persuasive. Fortunately, for a thermodynamic analysis of the dependence of catecholamine gradients on ΔpH and Δψ, it is not necessary to discriminate among these species. Consider the species E^m bearing a net charge m and assume that it is exchanged for $n + m$ H^+ ions. The equilibrium gradient for that species will be

$$\frac{[E^m]_{in}}{[E^m]_{out}} = \left(\frac{[H^+]_{in}}{[H^+]_{out}}\right)^{n+m} e^{nF\Delta\psi/(RT)} \quad (4)$$

At physiological pH, the total catecholamine concentration is essentially the concentration of the cationic species EH_2^+. Consequently, the equilibrium in eq 4 must be amended to include the equilibria between E^m and EH_2^+ in both internal and external media. When this is done, the four possible catecholamine/H^+ exchanges (Figure 3) all predict the same equilibrium gradient for the predominant EH_2^+ species:

$$\frac{[EH_2^+]_{in}}{[EH_2^+]_{out}} = \left(\frac{[H^+]_{in}}{[H^+]_{out}}\right)^{n+1} e^{nF\Delta\psi/(RT)} \quad (5)$$

Notice that if $n = 0$ eq 5 reduces to the weak base equilibrium

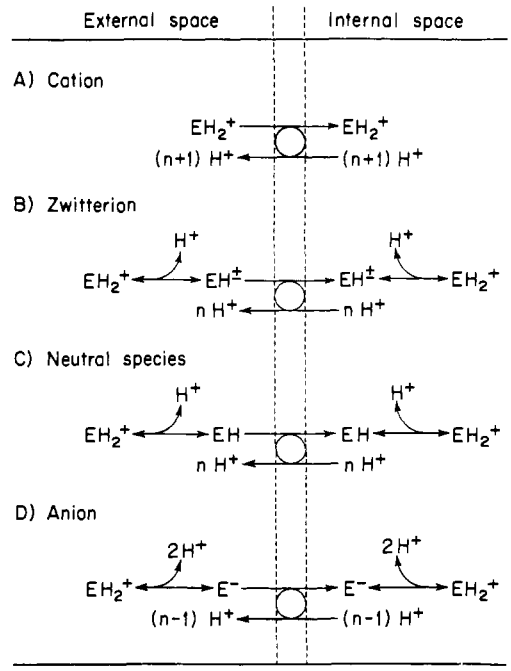

FIGURE 3: H^+/epinephrine exchange mechanisms for different permeant species.

(eq 3). As discussed before, this cannot account for ATP-dependent epinephrine gradients as large as those observed. If n is taken as 1, the measured epinephrine gradients are within the limit allowed by eq 5 (Table IV). Consequently, $n = 1$ appears to be a reasonable stoichiometry for the epinephrine/H^+ exchange. This would imply that 2 H^+ must be exchanged for each catecholamine cation (Figure 3A) or that 1 H^+ must be exchanged for each neutral or zwitterionic catecholamine (parts B and C of Figure 3).

The above rationalization of a stoichiometry of $n = 1$ is not entirely satisfactory because it relies on accurate knowledge of $[E]_{in}/[E]_{out}$, Δψ, and ΔpH. These can be distorted by error in measuring the internal volume. Moreover, we have not measured $[E]_{in}/[E]_{out}$ at equilibrium. Epinephrine uptake is slow and continues for 1 h or more. After this length of time, the ghosts deteriorate, Δψ decreases, and scatter in the measurements increases. This makes it very difficult to tell if $[E]_{in}/[E]_{out}$ is really ever at equilibrium with the existing Δψ and ΔpH.

A more satisfactory method of determining the stoichiometry n is provided by the plot in Figure 1. Since SCN^- distributes according to the Nernst equation (eq 2), eq 5 can be expressed as

$$\ln\frac{[E]_{in}}{[E]_{out}} = (n+1)\ln\frac{[H^+]_{in}}{[H^+]_{out}} + n\ln\frac{[SCN^-]_{in}}{[SCN^-]_{out}} \quad (6)$$

Since proton pumping in sucrose medium does not change $[H^+]_{in}$ or $[H^+]_{out}$, those terms are constants. Therefore, if ln $([SCN^-]_{in}/[SCN^-]_{out})$ is plotted against ln $([E]_{in}/[E]_{out})$, the slope will be n. This, of course, applies to equilibrium, but the requirement for equilibrium can be relaxed under certain conditions. If varying the membrane potential changes the equilibrium value of $[E]_{in}$ ($[E]_{in}^{eq}$) and the observed value of $[E]_{in}$ ($[E]_{in}^{ob}$) in the same proportion, then $[E]_{in}^{eq} = C[E]_{in}^{ob}$, C being a constant. When $C[E]_{in}^{ob}$ is substituted into eq 6, ln C becomes an added constant affecting the intercept of the line but not the slope. This means that the slope will be equal to n even during the period before catecholamine uptake reaches equilibrium. The conditions for relaxing the equi-

librium requirement are met if the slope does not vary with the incubation time. The average slope measured after 60 min was 1.0 ± 0.2. We have obtained slopes of ~1 for incubation times as short as 20 min (J. Knoth, K. Handloser, and D. Njus, unpublished observations). This confirms that the stoichiometry n of catecholamine/H^+ exchange is ~1. Errors in estimating the internal volume do not affect this measurement of the stoichiometry because they distort all internal concentrations by the same factor. Again, when the natural logarithm is taken, this factor becomes an added constant which does not affect the slope. Because the slope in Figure 1 is not sensitive to error in measuring internal volume and does not depend on equilibrium epinephrine gradients, it provides the most satisfactory measure of the catecholamine/H^+ stoichiometry.

Johnson et al. (1978) and Holz (1979) have noted that, in the absence of proton pumping, catecholamine distributions in intact chromaffin granules appear to follow a weak base equilibrium (eq 3). Our results show that the same seems to be true in uncoupled ghosts (Figure 2). Although both Johnson et al. and Holz suggested that different mechanisms may operate in active and passive conditions, the mechanism described by eq 5 provides a unified explanation for both. In the resting state, $\Delta\psi$ is determined by the H^+ diffusion potential (Pollard et al., 1976; Holz, 1979; Johnson & Scarpa, 1979):

$$\frac{[H^+]_{out}}{[H^+]_{in}} = e^{F\Delta\psi/(RT)} \quad (7)$$

Substitution of eq 7 into eq 5 gives the weak base equilibrium (eq 3). Put another way, the uncoupled membrane potential $\Delta\psi$ is equal and opposite to the H^+ concentration gradient. Since, in eq 5, the concentration gradient is raised one power higher than the membrane potential term, $\Delta\psi$ cancels all but one power of the H^+ concentration gradient. Therefore, in uncoupled or resting ghosts, the catecholamine gradient is equal to the H^+ gradient, and the electrogenic mechanism described by eq 5 is indistinguishable from the weak base equilibrium. It is possible to discriminate between the two cases only when proton pumping is occurring. This may be responsible for much of the confusion surrounding the electrogenic nature of catecholamine transport.

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