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# A Potassium Ion Diffusion Potential Causes Adrenaline Uptake in Chromaffin-Granule 'Ghosts'

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Membrane vesicles ('ghosts') formed from bovine chromaffin granules accumulate adrenaline in response to a diffusion potential produced by adding $K^+$ in the presence of valinomycin. This uptake occurs as a short (2–5 min) burst because of the transient nature of the diffusion potential. The potential-driven uptake is optimal at pH$\sim$7.2, is inhibited by reserpine, and has an initial rate comparable with that of ATP-driven uptake. These results show that ATP-dependent adrenaline uptake may occur at least partly in response to the membrane potential generated by an electrogenic proton-translocating adenosine triphosphatase found in chromaffin-granule membranes.

Chromaffin granules, the catecholamine-storage vesicles of the adrenal medulla, accumulate catecholamines via an ATP-requiring process (Kirshner, 1962). The granule membranes contain an inwardly directed proton-translocating ATPase (Casey *et al.*, 1977; Njus *et al.*, 1978) that can generate transmembrane gradients in pH (acid inside) or electrical potential (positive inside). Uncouplers of oxidative phosphorylation abolish catecholamine uptake (Bashford *et al.*, 1975*b*), indicating that catecholamine transport is linked to the proton-translocating ATPase (Njus & Radda, 1978). The ATPase pumps protons into the granules; obligatory exchange of catecholamines for protons then results in catecholamine uptake. The catecholamine/$H^+$ exchange must involve a net transfer of protons, so transport should respond to the pH gradient across the membrane. To elucidate the catecholamine-transport mechanism, it is important to know whether the exchange also results in a net transfer of charge. If so, then catecholamine uptake will be driven by the membrane potential.

Active proton translocation generates pH gradients across the chromaffin-granule membrane when the granules are suspended in media containing a permeant anion (Casey *et al.*, 1977). When permeant anions are absent, the proton pump generates a membrane potential, but does not affect the pH gradient. Because catecholamine uptake occurs in media lacking a permeant anion, we (Casey *et al.*, 1977; Njus & Radda, 1978) have argued that membrane potential must be capable of driving catecholamine uptake. Supporting this view, Holz (1978) has reported that increasing concentrations of uncouplers inhibit catecholamine uptake and ATP-dependent membrane potential in parallel. Nevertheless, Phillips (1978), Johnson *et al.* (1978) and Schuldiner *et al.* (1978) have argued that the uptake mechanism is electroneutral; it responds to pH gradients, but should not respond to membrane potential.

To resolve this question, it is necessary to show whether or not catecholamine uptake occurs in response to an imposed membrane potential. The ionophore valinomycin facilitates electrogenic translocation of $K^+$ across lipid membranes (Henderson *et al.*, 1969). Therefore, when a potassium salt is added to the solution on the outside of a vesicle, valinomycin will permit $K^+$ to cross the membrane. If the anion is impermeant, the selective translocation of $K^+$ will generate a diffusion potential with the electrical potential positive inside relative to outside. Such artificially generated membrane potentials have been shown to cause accumulation of amino acids and sugars in *Escherichia coli* membrane vesicles (Hirata *et al.*, 1974) and $Na^+$-dependent glucose transport in intestinal brush-border membranes (Murer & Hopfer, 1974). We now report here on catecholamine uptake into chromaffin-granule 'ghosts' in response to valinomycin-induced $K^+$ diffusion potentials.

## Experimental

Chromaffin granules were prepared from bovine adrenal medullae as described by Kirshner (1962) except that 0.3M-sucrose/10mM-Hepes, pH 7.0, was used as the isolation medium. 'Ghosts' were prepared by resuspending the granules in 0.2M-Tris/phosphate, pH 7.0, and adding $\frac{1}{8}$ vol. of glycerol/0.2M-Tris/phosphate, pH 7.0 (3:7, v/v). Granules were left to lyse for 15 min at 4°C and then pelleted by centrifugation at 36000*g* for 20 min at 4°C. The membranes were resuspended in 0.2M-Tris/phosphate, pH 7.0, and 15 ml samples were layered on discontinuous density gradients over 5 ml of 15% Ficoll,

Abbreviations used: ATPase, adenosine triphosphatase; Hepes, 4-(2-hydroxyethyl-1-piperazine-ethanesulphonic acid; Mes, 4-morpholine-ethanesulphonic acid.

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85% 0.3M-sucrose/10mM-Hepes, pH7.0 (w/w), and 10ml of 0.4M-sucrose/10mM-Hepes, pH7.0. After centrifugation at 25000 rev./min (90000$g$) for 30min at 4°C in an SW 25.3 rotor of a Beckman L2-65B ultracentrifuge, bands of 'ghosts' were collected. All experiments were performed within 24h of the cattle being slaughtered. The 'ghosts' obtained gave an oligomycin-insensitive ATPase activity and were similar to those, obtained by related methods (Phillips, 1974*a*; Bashford *et al.*, 1975*b*; Buckland *et al.*, 1978), that have been shown to be free of mitochondrial contamination. Other properties of the 'ghosts' prepared by this slightly modified procedure are also indicative of lack of mitochondrial interference (Drake *et al.*, 1979).

The time-course of $K^+$/valinomycin-dependent adrenaline uptake was followed by periodically collecting samples of 'ghosts' in Millipore filters (Phillips, 1974*a*). Chromaffin-granule 'ghosts' (1ml) were added to 10ml of buffer. Then 25$\mu$l of 200$\mu$M-valinomycin (in ethanol) and 100$\mu$l of 5mM-[$^3$H]adrenaline (50$\mu$Ci/ml in 50mM-acetic acid) were added. The 'ghosts' were incubated at 35–37°C for about 30min to allow passive catecholamine uptake to proceed to equilibrium. Two 1ml samples were then collected on Millipore filters (0.45$\mu$m pore size) and washed with about 2ml of cold buffer. At zero time, 0.8ml of 0.5M-$K_2SO_4$ was added to the remaining incubation mixture and 1ml samples were collected periodically. Uptake in these latter samples was multiplied by 1.09 to correct for the dilution resulting from $K_2SO_4$ addition. Experiments were always done in duplicate and were also repeated with different 'ghost' preparations. The results represent single experiments. Since each experiment contains a series of points often collected at slightly different times, the results do not lend themselves to error analysis. In all cases the duplicate curves were very similar in shape.

The time-course of ATP-dependent adrenaline uptake (Fig. 5) was followed in a similar way except that valinomycin was omitted and 0.4ml of 100mM-$MgSO_4$/100mM-ATP, pH7, was added in place of $K_2SO_4$.

Buffers were adjusted to the specified pH by using Tris base to avoid introducing unnecessary $K^+$ or $Na^+$ ions. 8-Anilinonaphthalene-1-sulphonic acid fluorescence was monitored at 480nm on a Hitachi–Perkin–Elmer MPF4A spectrofluorimeter with the excitation wavelength set at 380nm. Protein was assayed by using the biuret method as described by Casey *et al.* (1976).

### *Materials*

Valinomycin was obtained from Eli Lilly and Co., Indianapolis, IN, U.S.A. DL-[7-$^3$H]Adrenaline hydrochloride was obtained from The Radiochemical Centre, Amersham, Bucks., U.K. Reserpine, disodium ATP, Hepes, Mes, Tris and unlabelled adrenaline were obtained from Sigma Chemical Co., Poole, Dorset, U.K. 8-Anilinonaphthalene-1-sulphonic acid was obtained from K & K Laboratories (via Kodak, Kirkby, Liverpool, U.K.) and recrystallized as the magnesium salt before use.

## Results

If a salt concentration gradient exists across a membrane and the membrane is made permeable to either the anion or the cation but not both, a diffusion potential will arise. Valinomycin facilitates electrogenic $K^+$ transfer across membranes (Henderson *et al.*, 1969) and thus allows the creation of a $K^+$ diffusion potential. If $K^+$ is in higher concentration on the outside of a vesicle membrane, the electrical potential will be positive inside relative to outside. Consequently, in chromaffin-granule 'ghosts', the membrane potential will be in the same sense as that created by the ATPase-driven proton pump.

Like the enzymically generated membrane potentials, diffusion potentials enhance the fluorescence of 8-anilinonaphthalene-1-sulphonic acid, an extrinsic probe (Jasaitis *et al.*, 1971; Bakker & Van Dam, 1974). Consequently, 8-anilinonaphthalene-1-sulphonic acid fluorescence provides us with a convenient way of following the qualitative behaviour of valinomycin-induced $K^+$ diffusion potentials. As shown by Bashford *et al.* (1976), when chromaffin granules are suspended in a medium containing 50mM-$K^+$ at 25°C valinomycin causes a rapid fluorescence enhancement followed by a first-order fluorescence decay. As shown in Fig. 1, a similar response is seen when $K_2SO_4$ (final concn. 40mM) is added to a suspension of chromaffin-granule 'ghosts' at 37°C in the presence of valinomycin. (To avoid premature dissipation of the $K^+$ gradients, we initiated diffusion potentials by adding $K^+$ after valinomycin rather than vice versa.) The fluorescence enhancement confirms that the diffusion potential is in the same sense as the membrane potential created by ATP hydrolysis. The rapid decay indicates that the diffusion potential is very transient, lasting only 2–5min.

The diffusion potential should not arise if valinomycin is omitted because the chromaffin-granule membrane itself is impermeable to $K^+$ (Johnson & Scarpa, 1976*b*). Valinomycin transports $K^+$ much more readily than $Na^+$ (Henderson *et al.*, 1969), so $Na_2SO_4$ should not support a diffusion potential even in the presence of valinomycin. Finally, because $SCN^-$ is a permeant anion (Dolais-Kitabgi & Perlman, 1975; Casey *et al.*, 1976), KSCN cannot be used to produce a $K^+$ diffusion potential. The expectation that diffusion potentials should not arise if valinomycin is omitted or if $Na_2SO_4$ or KSCN is added instead of

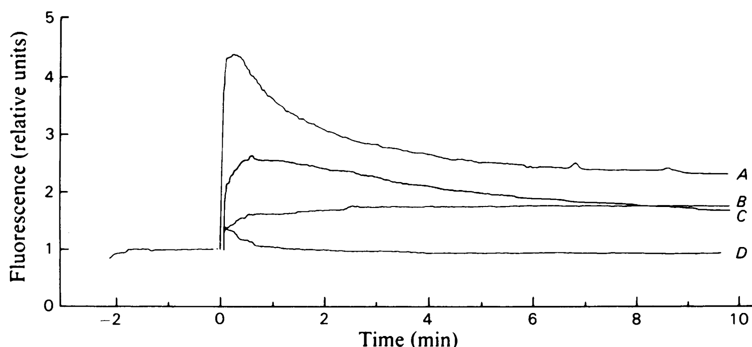


Fig. 1. *Fluorescence of 8-anilinonaphthalene-1-sulphonate stimulated by a diffusion potential*
For each trace, 50μl of 'ghosts' (0.44mg of protein) was added to 2ml of 0.4M-sucrose/40mM-Hepes, pH7.5; 5μl of 200μM-valinomycin in ethanol was added to samples *A*, *C* and *D*, and 5μl of ethanol was added to sample *B*. After incubation at 37°C for about 20min, 10μl of 1mM-8-anilinonaphthalene-1-sulphonate was added to each sample and the fluorescence was followed as described in the Experimental section. At $t = 0$ the following were added : *A* and *B*, 0.2ml of 0.5M-$K_2SO_4$; *C*, 0.2ml of 0.5M-$Na_2SO_4$; *D*, 0.2ml of 1.0M-KSCN.

$K_2SO_4$ is confirmed by the fact that 8-anilinonaphthalene-1-sulphonic acid responses are not seen under these conditions (Fig. 1).

The diffusion potential is short-lived, whereas the enzymically generated potential lasts until the ATP is exhausted. The ATP-driven 8-anilinonaphthalene-1-sulphonic acid response persists for 1h or more (Bashford *et al.*, 1975*a*), and ATP-driven catecholamine uptake proceeds for 30–60min (Phillips, 1974*b*; Bashford *et al.*, 1975*b*). In order to study the response of catecholamine uptake to transient membrane potentials, the following strategy was adopted; 'ghosts' were incubated for 30min in the presence of [$^3$H]adrenaline and valinomycin to allow the catecholamine to equilibrate across the membrane in accord with the pH gradient. At this point, $K_2SO_4$ (final conc. 41mM) was added to initiate the membrane potential without changing the pH gradient, and catecholamine uptake was followed. $K_2SO_4$ addition did cause catecholamine uptake (Fig. 2). That this is indeed driven by the diffusion potential and not simply by salt addition is indicated by the fact that uptake is not observed if valinomycin is omitted or if $Na_2SO_4$ or KSCN is added instead of $K_2SO_4$.

This uptake is inhibited by 9.1μM-reserpine (Fig. 3). Reserpine, which inhibits ATP-driven catecholamine uptake (Kirshner, 1962; Phillips, 1974*a*), but does not inhibit ATPase activity (Bashford *et al.*, 1975*a*), is thought to specifically block the catecholamine carrier. Reserpine does not affect the 8-anilinonaphthalene-1-sulphonic acid response to the valinomycin-induced $K^+$ diffusion potential. Because chromaffin-granule ATPase activity reaches a maximum at pH6.6 (Bashford *et al.*, 1975*a*), whereas ATP-driven catecholamine uptake is maximal at pH7.4 (Taugner, 1972), the transport system is assumed to have a pH optimum at alkaline pH. The $K^+$/valinomycin-dependent uptake is maximal at pH~7.2 (Fig. 4). These similarities in reserpine-sensitivity and pH profile indicate that uptake driven by a diffusion

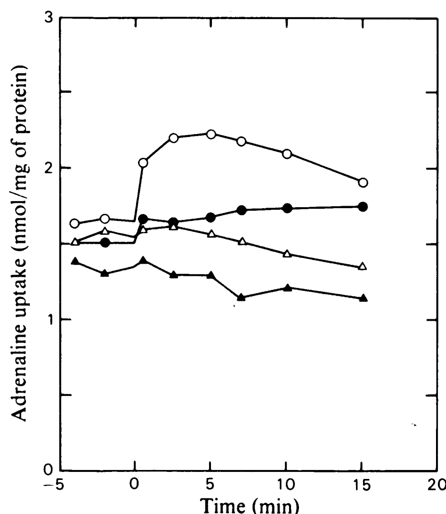


Fig. 2. *Adrenaline uptake stimulated by a diffusion potential*
The time course of $K^+$/valinomycin-dependent uptake was followed as described in the Experimental section. For each series of points 1ml of 'ghosts' (9.5mg of protein) was added to 10ml of 0.4M-sucrose/40mM-Hepes, pH7.5. Then 30min before $t = 0$ valinomycin and [$^3$H]adrenaline were added and incubation at 36°C was begun. One sample (●) contained 20μl of ethanol instead of valinomycin. After two assays of unstimulated uptake, one of the following was added at $t = 0$: 0.8ml of 0.5M-$K_2SO_4$ (○ and ●); 0.8ml of 0.5M-$Na_2SO_4$ (△); 0.8ml of 1M-KSCN (▲).

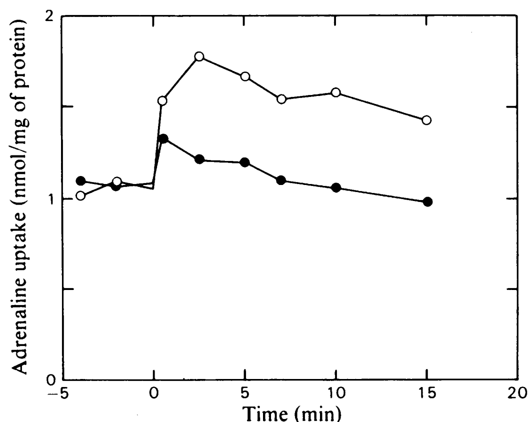


Fig. 3. *Inhibition of stimulated adrenaline uptake by reserpine.*

For each time course 1ml of 'ghosts' (7.9mg of protein) was added to 10ml of 0.4M-sucrose/40mM-Hepes, pH7.5. Then 35min before $t = 0$ valinomycin and [$^4$H]adrenaline were added and incubation at 36°C was begun. At $t = -9$min the sample received either 20$\mu$l of 5mM-reserpine in acetone/ethanol (3:2, v/v) (●) or 20$\mu$l of solvent alone (○). Unstimulated uptake was assayed twice and 0.8ml of 0.5M-$K_2SO_4$ was added to $t = 0$.

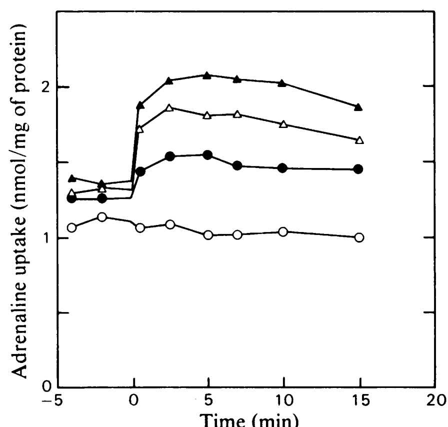


Fig. 4. *pH-dependence of stimulated adrenaline uptake*

For each series of points 1ml of 'ghosts' (9.3mg of protein) was added to 10ml of buffer and the time course of $K^+$/valinomycin-dependent uptake was followed as described in the Experimental section. During the incubation period before $K_2SO_4$ addition (30min at 35°C), the pH was measured. Buffers were: ○, 0.4M-sucrose/40mM-Mes, pH6.26; ●, 0.4M-sucrose/40mM-Mes, pH6.78; ▲, 0.4M-sucrose/40mM-Hepes, pH7.15; △, 0.4M-sucrose/40mM-Hepes, pH7.68.

potential uses the same transport system as uptake driven by ATP hydrolysis.

Because ATP-dependent catecholamine uptake persists for 1h, uptake reaches a far higher value than that obtained with the short-lived diffusion potential. However, the initial rates of uptake obtained with ATP and with the diffusion potential are comparable (Fig. 5).

## Discussion

We have shown that a $K^+$ diffusion potential drives adrenaline uptake into chromaffin-granule 'ghosts'. That the diffusion potential causes uptake indirectly by changing the pH gradient across the membrane is unlikely. The diffusion potential makes the granule interior more positive, so protons should tend to be expelled, and the pH inside, if anything, should rise. Phillips (1978) and Schuldiner *et al.* (1978) have shown that an increase in internal pH inhibits uptake rather than promotes it.

The possibility that adrenaline uptake is only apparent and that the observed effect merely represents a diffusion potential dependent binding of catecholamine to the outside of the membrane can be excluded for the following reasons. (i) The pH-dependence of the process is similar to that of the ATP-driven uptake. (ii) Reserpine is inhibitory. (One should note,

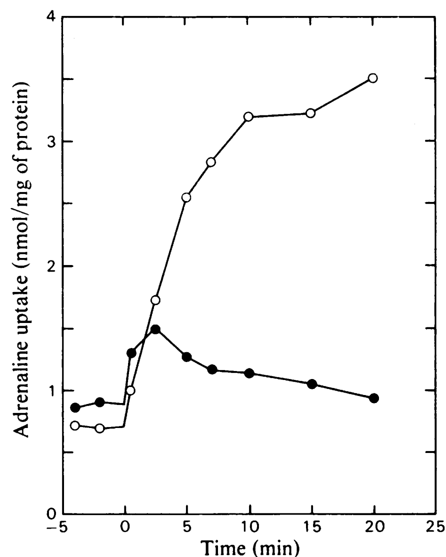


Fig. 5. *Comparison of $K^+$/valinomycin-dependent and ATP-dependent adrenaline uptake*

For each trace 1ml of 'ghosts' (13.2mg of protein) was added to 10ml of 0.4M-sucrose/40mM-Hepes, pH7.4. The time course of $K^+$/valinomycin-dependent (●) and ATP-dependent (○) uptake were followed as described in the Experimental section. Incubation before $K_2SO_4$ or ATP addition was for 30min at 36°C.

however, that although the specific reserpine effect is generally taken as evidence for a carrier-mediated transport, it is by no means conclusive.) (iii) The extent of the diffusion-potential-dependent uptake, though small, can be as much as one-third of the ATP-driven uptake. (iv) A potential, positive inside, leads to increased binding of negative ions (as is shown by the response of 8-anilinonaphthalene-1-sulphonate) and to the opposite effect on positively charged molecules.

The fact that a $K^+$ diffusion potential drives catecholamine uptake in chromaffin-granule membranes demonstrates that this transport is electrogenic. Catecholamine uptake results in a net exit of positive charge from the 'ghosts'. Further evidence for this assertion is the report by Holz (1978) that increasing concentrations of the uncoupler carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone inhibit the membrane potential and catecholamine uptake in parallel. Finally, we have noted before (Casey *et al.*, 1977; Njus & Radda, 1978) that ATP hydrolysis does not affect the pH gradient when chromaffin granules are suspended in a medium free of permeant anions, but it stimulates catecholamine uptake nevertheless. This implies that uptake is driven by the ATP-generated membrane potential.

Despite this evidence, several investigators (Phillips, 1978; Johnson *et al.*, 1978; Schuldiner *et al.*, 1978) have argued that catecholamine transport is electroneutral. These ideas are based on the appealing notion that catecholamines equilibrate across the membrane as weak bases. Weak bases ($RNH^+$) cross lipid membranes in their neutral form, but not in their protonated form, and hence equilibrate across vesicle membranes according to the pH gradient:

$$\frac{[RNH^+]_{in}}{[RNH^+]_{out}} = \frac{[H^+]_{in}}{[H^+]_{out}}$$

For this reason, weak bases tend to accumulate inside acidic vesicles. Pursuing this idea, Nichols & Deamer (1976) showed that acid-loaded phosphatidylcholine liposomes accumulate catecholamines. Because intact chromaffin granules have an acidic internal pH, they take up high concentrations of the weak base methylamine (Johnson & Scarpa, 1976*a*; Pollard *et al.*, 1976; Casey *et al.*, 1977), and it is tempting to think that catecholamines accumulate inside the granules by this sort of mechanism.

We have pointed out before that this simple equilibration across lipid membranes is unlikely to account for transport in chromaffin granules, because catecholamine uptake seems to occur via a carrier (Njus & Radda, 1978). This is indicated by the observations that uptake is inhibited by the specific inhibitor reserpine, uptake exhibits saturation kinetics, and uptake is stereospecific. Of course, a carrier mechanism may simply facilitate the equilibration of the neutral form of the catecholamine, so it does not preclude the possibility of a weak-base equilibrium.

Two groups (Phillips, 1978; Schuldiner *et al.*, 1978) have shown that catecholamines are taken up by chromaffin-granule 'ghosts' if a pH gradient is imposed across the membrane, and have argued that uptake must occur via an electroneutral (weak-base) mechanism. However, electrogenic exchanges of catecholamines and protons will depend on the proton gradient as well as on the membrane potential.

It is true that electrogenic uptake is not expected to proceed freely, because such uptake will generate a self-inhibitory membrane potential. Nevertheless some uptake may occur because fluxes of permeant ions neutralize the membrane potential. In the experiments reported by Schuldiner *et al.* (1978), the permeant $Cl^-$ anion was present on both sides of the 'ghost' membrane and small $Cl^-$ fluxes would tend to dissipate the membrane potential, allowing electrogenic uptake to occur. In the experiments reported by Phillips (1978), permeant anions added to the external medium inhibited uptake. Of course, this addition will set up a diffusion potential. Just as a $K^+$ diffusion potential stimulates uptake, an anion diffusion potential would be expected to inhibit uptake, but only if the uptake mechanism is electrogenic.

Phillips (1978) also reported that the uncoupler carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone inhibits uptake caused by a change in external pH. Uncouplers will inhibit uptake occurring by an electroneutral mechanism only if they dissipate the pH gradient. In general, uncouplers do not dissipate the pH gradient, but allow protons to flow across the membrane only until the charge flow generates a membrane potential that exactly balances the existing pH gradient. In fact, Phillips's (1978) data show that, in 'ghosts' energized with ATP, the uncoupler does not materially affect the pH gradient, but substantially diminishes the membrane potential. This result too supports the idea of an electrogenic transport mechanism.

Johnson *et al.* (1978) found that adding high concentrations of dopamine (3,4-dihydroxyphenethylamine) to intact chromaffin granules causes the internal pH to rise. This is similar to the effect seen when a weak base such as $NH_3$ or methylamine is added to the granular suspension. The weak base penetrates the membrane as the neutral species and is reprotonated once inside, thus removing $H^+$ from the internal medium. If dopamine uptake were electrogenic, one would not expect dopamine uptake to be sufficient to change the internal pH, since it would be limited by a membrane potential.

It is possible that adrenaline uptake is electrogenic and that dopamine uptake is not. However, ATP does drive dopamine uptake in media lacking a permeant anion (Bashford *et al.*, 1976), conditions in which the pH gradient is not affected. This suggests that ATP-

driven dopamine uptake is driven by the membrane potential. Johnson *et al.* (1978) recognize this fact and suggest that ATP-driven uptake may be electrogenic, although an electroneutral pathway also exists. We do not favour this hypothesis, since such a mechanism would seem to uncouple the proton pump. The co-existence of a neutral and an electrogenic pathway would enable catecholamines to shuttle back and forth across the membrane via different pathways, causing a net proton efflux.

Johnson *et al.* (1978) noted that, when the membrane pH gradient is dissipated with a weak base such as $NH_3$ or methylamine, ATP addition does not reverse the effect. This is to be expected, since proton pumping will be limited by the generation of a membrane potential in the absence of a permeant anion. However, Johnson *et al.* (1978) found that, when the pH gradient is dissipated by dopamine addition, ATP does restore the pH gradient. The fact that an electrogenic proton pump can restore the pH gradient under these conditions is strange and casts doubt on the previous assumption that the pH gradient is created by electroneutral dopamine uptake. Other explanations for the dopamine effect on the pH gradient should be sought. For example, since Johnson *et al.* (1978) are working with intact granules rather than 'ghosts', it is possible that dopamine is not actually being taken up, but is exchanging across the membrane for intragranular adrenaline and ATP.

We (Casey *et al.*, 1976) and Dolais-Kitabgi & Perlman (1975) have shown that sulphate is an impermeant anion, and this has been an important premise in much recent work on chromaffin granules. Phillips & Allison (1978) have challenged this idea, arguing that $SO_4^{2-}$ is taken up by chromaffin-granule 'ghosts' via an $H^+/SO_4^{2-}$ symport. This was deduced from the observation that, when ATP is added to 'ghosts' suspended in $K_2SO_4$, the light-scattering of the suspension decreased, implying that the 'ghosts' were swelling. However, when 'ghosts' are energized in the presence of a permeant anion such as $Cl^-$, the $H^+/Cl^-$ influx driven by the proton pump causes a measurable change in intragranular pH (Casey *et al.*, 1977). Phillips & Allison (1978) observed a pH change of 1.53–1.69 across granule 'ghosts' in media containing $Cl^-$. In a medium containing 40mM-$SO_4^{2-}$ a pH gradient of 1.71–1.29 was observed, and a comparable pH gradient (1.26) was observed in sucrose (containing 3mM-$MgSO_4$). These data are hard to reconcile with the claims by Phillips & Allison (1978) that $H^+/SO_4^{2-}$ symport is significant, particularly since these authors themselves were only able to demonstrate 'marginal' $^{35}SO_4^{2-}$ incorporation. This implies that $H^+/SO_4^{2-}$ uptake is not significant and casts considerable doubt on the interpretation of the light-scattering experiments.

We have discussed before possible adrenaline-uptake mechanisms (Njus & Radda, 1978). To define the mechanism, it is necessary to exclude the possibility that anions, particularly ATP and its metabolic products, are co-transported with adrenaline. The fact that uptake stimulated by a diffusion potential is observed in the absence of ATP adds to the evidence that adrenaline uptake does not require co-transport of any other ions except $H^+$. We do not know whether the charged or the neutral form of adrenaline is transported. Since uptake is electrogenic, at least 1 $H^+$ ion would have to be released for each neutral catecholamine molecule taken up, and at least 2 $H^+$ ions would have to be exchanged for each catecholamine cation.

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## References

Bakker, E. P. & Van Dam, K. (1974) *Biochim. Biophys. Acta* **339**, 157–163

Bashford, C. L., Radda, G. K. & Ritchie, G. A. (1975*a*) *FEBS Lett.* **50**, 21–24

Bashford, C. L., Casey, R. P., Radda, G. K. & Ritchie, G. A. (1975*b*) *Biochem. J.* **148**, 153–155

Bashford, C. L., Casey, R. P., Radda, G. K. & Ritchie, G. A. (1976) *Neuroscience* **1**, 399–412

Buckland, R. M., Radda, G. K. & Shennan, C. D. (1978) *Biochim. Biophys. Acta* **513**, 321–337

Casey, R P., Njus, D., Radda, G. K. & Sehr, P. A. (1976) *Biochem. J.* **158**, 583–588

Casey, R. P., Njus, D., Radda, K. G. & Sehr, P. A. (1977) *Biochemistry* **16**, 972–977

Dolais-Kitabgi, J. & Perlman, R. L. (1975) *Mol. Pharmacol.* **11**, 745–750

Drake, R. A. L., Harvey, S. A. K., Njus, D. & Radda, G. K. (1979) *Neuroscience* in the press

Henderson, P. J. F., McGiven, J. D. & Chappell, J. B. (1969) *Biochem. J.* **111**, 521–535

Hirata, H., Altendorf, K. &. Harold, F. M. (1974) *J. Biol. Chem.* **249**, 2939–2945

Holz, R. W. (1978) *Proc Natl. Acad. Sci. U.S.A.* **75**, 5190–5194

Jasaitis, A. A., Kuliene, V. V. & Skulachev, V. P. (1971) *Biochim. Biophys. Acta* **234**, 177–181

Johnson, R. G. & Scarpa, A. (1976*a*) *J. Biol. Chem.* **251**, 2189–2191

Johnson, R. G. & Scarpa, A (1976*b*) *J. Gen. Physiol.* **68**, 601–631

Johnson, R. G., Carlson, N. J. & Scarpa, A. (1978) *J. Biol. Chem.* **253**, 1512–1521

Kirshner, N. (1962) *J. Biol. Chem.* **237**, 2311–2317

Murer, H. & Hopfer, U. (1974) *Proc. Natl. Acad. Sci. U.S.A.* **71**, 484–488

Nichols, J. W. & Deamer, D. W. (1976) *Biochim. Biophys. Acta* **455**, 269–271

Njus, D. & Radda, G. K. (1978) *Biochim. Biophys. Acta* **463**, 219–244

Njus, D., Sehr, P. A., Radda, G. K., Ritchie, G. A. & Seeley, P. J. (1978) *Biochemistry* **17**, 4337–4343
Phillips, J. H. (1974*a*) *Biochem. J.* **144**, 311–318
Phillips, J. H. (1974*b*) *Biochem. J.* **144**, 319–325
Phillips, J. H. (1978) *Biochem. J.* **170**, 673–679
Phillips, J. H. & Allison, Y. P. (1978) *Biochem. J.* **170**, 661–672
Pollard, H. B., Zinder, O., Hoffman, P. G. & Nikodijevic, O. (1976) *J. Biol. Chem.* **251**, 4544–4550
Schuldiner, S., Fishkes, H. & Kanner, B. I. (1978) *Proc. Natl. Acad. Sci. U.S.A.* **75**, 3713–3716
Taugner, G. (1972) *Naunyn-Schmiedeberg's Arch. Pharmacol.* **274**, 299–314