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CATECHOLAMINE UPTAKE AND CONCENTRATION BY LIPOSOMES MAINTAINING pH GRADIENTS

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Summary

Liposomes were prepared with pH gradients across their membranes (acidic interiors with respect to the external buffer). These liposomes efficiently concentrated several catecholamines (dopamine, norepinephrine, and epinephrine) added to the external buffer. Our observations support a mechanism which suggests that pH gradients may contribute to uptake of catecholamines by sub-cellular storage sites.

A pH gradient can profoundly influence the distribution of weak acids and bases across membranes. In past studies, this effect has been used to measure pH gradients in mitochondria [1] with a weak acid, and in chloroplasts [2], lysosomes [3], chromaffin granules [4] and liposomes [5] with amines such as methylamine and 9-aminoacridine. In the present study, using liposomes as a model system, we have tested the possibility that catecholamines may be concentrated by pH gradients.

Ideally, the charged form of the amine is assumed to be unable to penetrate a membrane while the uncharged form can freely penetrate. For this reason, equal concentrations of the uncharged form of the amine will exist across a membrane at equilibrium, while the charged form will be distributed in proportion to the difference in pH. Net distribution of the amine is described by the relationship

$$\frac{[A^T]_i}{[A^T]_o} = \frac{K_a + [H^+]_i}{K_a + [H^+]_o}$$

where $[A^T]_i$ and $[A^T]_o$ are the total internal and external concentrations of the amine, $[H^+]_i$ and $[H^+]_o$ are the internal and external proton concentrations, and K_a is the dissociation constant of the amine [2, 5]. It follows that a

pH gradient, acid inside, will drive accumulation of amines into the internal volume. We will show here that the distribution of catecholamines across liposome membranes maintaining a pH gradient is qualitatively similar to that which would be expected from the above equation.

Liposomes were prepared by injecting 7 ml of egg phosphatidylcholine dissolved in ether (2 μmol/ml) into 14 ml of citrate-phosphate buffer, pH 5.0 at 55°C. When injected under these conditions, the ether vaporizes and the phospholipid forms 0.05—0.2 μm diameter vesicles which efficiently trap buffer [6]. Liposome-buffer mixtures were filtered through a 1.2 μm millipore filter to remove a small fraction of larger, multilamellar liposomes. The liposome-buffer mixtures were titrated with NaOH to pH 8, establishing an initial gradient of 3 pH units. Tritium-labelled catecholamine (epinephrine, norepinephrine, dopamine) was then added to the liposome-buffer mixture to a final concentration of 10 μM (5000 cpm/0.1 ml). 2-ml aliquots of the liposome-buffer incubation mixture were withdrawn at timed intervals and filtered through a Sephadex G-50 column using a pH 8 citrate-phosphate buffer for elution. This procedure separated the liposomes containing catechol-

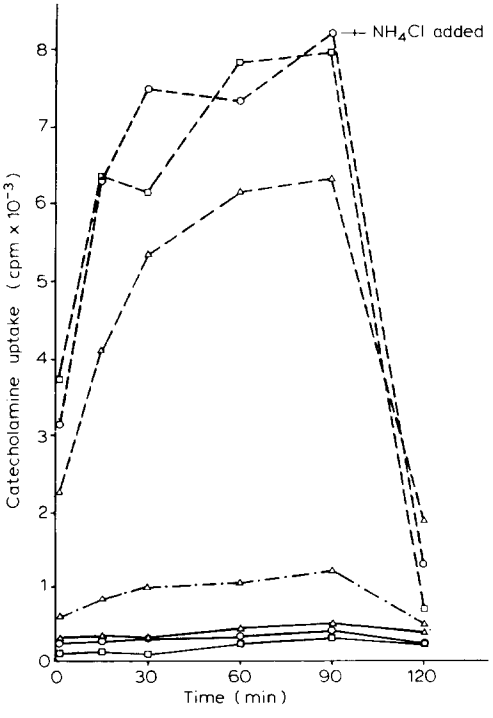

Fig. 1. Accumulation of catecholamines by liposomes maintaining three different pH gradients. Liposomes were prepared in citrate-phosphate buffer (pH 5.0). Liposome-buffer mixtures were titrated with NaOH to pH 8.0, establishing an initial gradient of 3 pH units. Tritium-labelled catecholamine was then added to the liposome-buffer mixture. Aliquots of the liposome-buffer incubation mixture were withdrawn at timed intervals. The liposomes were separated from the incubation buffer by filtering through a Sephadex G-50 column and counted. After 90 min, NH_4Cl was added to the remaining incubation mixture and a last sample was filtered at 120 min. — — —, pH gradient of 3 units; — · —, pH gradient of 1.5 units; ——, pH gradient of 0. △, [^{3}H]norepinephrine; □, [^{3}H]dopamine; ○, [^{3}H]epinephrine. Under similar conditions, no uptake of [^{14}C]acetylcholine could be detected.

amines from free catecholamines. After 90 min, 0.1 ml of 1 M NH_4Cl was added to the remaining incubation mixture and a last sample was filtered at 120 min. Addition of NH_4Cl destroys the pH gradient across the liposome membranes [5], permitting us to test for the pH dependence of any catecholamine accumulation. The tritium-labelled catecholamine trapped inside the liposome was counted on a Beckman CPM-100 liquid scintillation counter. Estimates of the degree of concentration were made by comparing the uptake in the presence of a pH gradient with that of controls in the absence of a pH gradient.

Under these conditions, we observed a remarkable accumulation of each of the catecholamines tested. Uptake into the liposomes occurred relatively slowly, and was maximal 90 min after addition of the amine. At this time we found concentrations of 12-fold over controls for norepinephrine. Epinephrine and dopamine were concentrated 18- and 23-fold respectively. In earlier experiments with Dr. Enrique Ochoa in 1975 (unpublished) we found that the indolamine serotonin was concentrated 40-fold over controls. When the gradients were destroyed by ammonium chloride additions, the accumulated catecholamines were released, demonstrating that the uptake was reversible and dependent upon pH gradients, Finally, when [^{14}C]acetylcholine was tested under similar conditions, no uptake was measureable.

It should be noted that the observed accumulation, 10—20-fold over controls, is only a fraction of that expected for an ideal monoamine responding to a 3 pH unit gradient. This is probably due to decay of the pH gradient over time. We have measured the decay rate in this system using 9-aminoacridine as a Δ pH indicator [5, 6] and found that half the pH gradient was lost in 30 min, in agreement with earlier results [6].

It is also interesting that the uptake of the catecholamines is relatively slow. For instance, the monoamine 9-aminoacridine reaches equilibrium within seconds under similar conditions [5]. The slow uptake is probably a function of the hydroxyl groups on the catecholamines, which would limit their permeability to lipid bilayer membranes.

These results suggest a potential role of pH gradients in the uptake and concentration of catecholamines by sub-cellular storage sites, as was suggested earlier by Johnson and Scarpa [4] for chromaffin granules. Our results are also consistent with Johnson and Scarpa's observation that chromaffin granules maintain acidic interiors (pH 5.5) with respect to a more alkaline external medium.

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