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Dopamine accumulation in large unilamellar vesicle systems induced by transmembrane ion gradients

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Transmembrane movement of dopamine in response to K^+ or H^+ ion gradients has been investigated. It is shown that dopamine can accumulate rapidly into large unilamellar vesicles (LUVs) composed of egg phosphatidylcholine exhibiting either a K^+ diffusion potential ($\Delta\psi$; negative inside) or a pH gradient (inside acidic). This can result in entrapped dopamine concentrations of 30—40 mM and inside-outside concentration gradients of nearly 300-fold. The transmembrane dopamine gradients formed in LUV systems exhibiting ΔpH (inside acidic) indicate that the transport process can be dictated by movement of the neutral form of dopamine which redistributes according to a simple Henderson-Hasselbach equilibrium. The mechanism of dopamine transport in response to a valinomycin-induced K^+ potential is more complex. Although generation of a K^+ diffusion potential results in acidification of the vesicle interior, the magnitude of the induced ΔpH (approx. 1 pH unit) is insufficient to account for the dopamine concentration gradient achieved (>200-fold). Further, data presented here suggest that higher uptake levels of dopamine can be achieved when certain anions (ATP and citrate) are entrapped within the LUV system. These anions may complex with the protonated form of dopamine creating a non-equilibrium trapping phenomena resulting in interior concentrations of dopamine in excess of that predicted by a simple Henderson-Hasselbach equilibrium.

Keywords: Dopamine; Ion gradients; Large unilamellar vesicles.

Introduction

The uptake of biogenic amines into secretory vesicles is generally thought to involve specific transport proteins [1,2]. It has been reported by Nicholls and Deamer [3], however, that dopamine and other catecholamines can be accumulated inside model membrane vesicle systems in response to a proton gradient (acid interior). Ion gradients are also present across membranes of secretory vesicles [4,5] and presumably target cell plasma membranes, leading to the interesting possibility of protein-independent uptake of biogenic amines.

We have previously shown that a variety of amino-containing weak bases can be accumulated in model large unilamellar vesicle (LUV) systems in response to a K^+ diffusion potential (negative inside) [6]. It was concluded from these studies that the positively charged (protonated) form of the amine could be transported in response to a transmembrane electrical gradient. This mechanism of amine transport, however, is not consistent with the concept that lipid bilayers are relatively impermeable to the charged (protonated) form of basic amines [7]. As suggested by Nicholls and Deamer [3], weak bases such as dopamine permeate across membranes in a neutral form and the resulting redistribution across the lipid bilayer is dictated by a simple Henderson-Hasselbach equilibrium. In order to more fully understand the possible effects membrane potentials and pH gradients may have on the transmembrane distribution of biogenic amines, we examine here the influence of K^+ and H^+ diffusion potentials on the uptake of dopamine into model LUV systems. We show that a valinomycin-induced K^+ diffusion poten-

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tial (negative inside) or a pH gradient (acid interior) can drive the rapid uptake of dopamine into LUVs to achieve interior to exterior concentration gradients of greater than 250. Mechanistically, transport of dopamine in response to ΔpH can be accounted for by redistribution of the neutral form of the amine. Surprisingly, however, the high uptake levels achieved in response to $\Delta\psi$ cannot simply be attributed to the formation of a $\Delta\psi$-induced pH gradient.

Materials and methods

Egg phosphatidylcholine (EPC) was isolated from hen egg yolks employing standard procedures. Dopamine, epinephrine and serotonin (5-hydroxytryptamine) were obtained from Sigma, St. Louis, MO. Radiolabelled [^{3}H]inulin, [^{14}C]cholesteryl oleate, [^{3}H]dopamine and [^{3}H]epinephrine were obtained from NEN.

Large unilamellar vesicles (LUVs) were prepared employing the LUVET (large unilamellar vesicles by extrusion techniques) procedure detailed elsewhere [8]. Briefly, EPC was deposited as a dry lipid film by evaporation from $CHCl_3$ under a stream of nitrogen, stored under vacuum for 2 h and hydrated with the appropriate buffer by vortex mixing to produce multilamellar vesicles (MLVs) at lipid concentrations of 25—100 μmol/ml. These MLVs were then extruded under nitrogen pressure (≤500 lb/inch^2) ten times through two (stacked) Nucleopore polycarbonate filters (pore size 100 nm). This preparation was then freeze-thawed twice (employing liquid nitrogen) and subsequently extruded a further five times through two stacked filters (100 nm pore size). The resulting preparation was unilamellar as indicated by ^{31}P-NMR techniques [8] with an average diameter of approximately 90 nm as determined by freeze-fracture techniques [8] and exhibited trapped volumes of 1.5 μl/μmol phospholipid determined employing [^{3}H]inulin as an aqueous marker.

Transmembrane Na^+-K^+ chemical gradients (K^+ inside) were obtained by first preparing EPC LUVETs in a K^+ buffer (169 mM potassium glutamate (K-Glu), 20 mM Hepes, adjusted to pH 7.5 employing NaOH, 310 mOsm/kg). Untrapped buffer was then removed by passing the LUVs through a Sephadex G-50 column pre-equilibrated with a NaCl buffer (150 mM NaCl, 20 mM Hepes, adjusted to pH 7.5 employing NaOH). Alternatively, in order to generate transmembrane pH gradients, the LUVs were prepared at low pH (175 mM glutamic acid 150 mM KOH (pH 4.65) 290 mOsm/kg) and untrapped buffer was removed employing a Sephadex G-50 column equilibrated with a neutral pH buffer (125 mM glutamic acid, 150 mM KOH, 30 mM NaCl (pH 7.5) 290 mOsm/kg). Other defined transmembrane pH gradients were generated by trapping buffers containing 10 mM MES (pK_a = 6.15), 10 mM Hepes (pK_a = 7.0), 10 mM glutamic acid (pK_a = 4.2) and 125 mM Na_2SO_4 adjusted to the desired pH. Subsequently, the vesicles were passed down Sephadex G-50 columns pre-equilibrated with the same buffer adjusted to pH 7.5. A similar buffering mixture (glutamic acid, MES, EPPS) was utilized when the pH was varied under conditions of a constant Na^+/K^+ ion gradient (interior K^+). Where ATP was utilized as an internal trap, the trapped buffer consisted of 150 mM KOH, 175 mM glutamic acid and 125 mM ATP (Na^+ salt) giving a pH of 4.3 (470 mOsm/kg). This was exchanged for either a buffer containing 150 mM KCl, 20 mM glutamic acid, and 80 mM NaCl (adjusted to pH 4.3 with 1 M NaOH; 470 mOsm/kg) for control samples or for a buffer containing 220 mM NaCl and 20 mM Hepes (adjusted to pH 7.5 with 1 M NaOH; 450 mOsm/kg). Where citrate was utilized as an internal trap, the trapped buffer was composed of 300 mM citrate which was adjusted to pH 4.0 with NaOH. All biogenic amine uptake studies were performed with buffers containing 5 mM sodium ascorbate as an anti-oxidant. Where employed, the K^+ ionophore valinomycin was added to give a final concentration of 0.5 μg/μmol lipid and the H^+ ionophore CCCP was added to give a final concentration of 20 μM.

Membrane potentials were determined by measuring the equilibrium transbilayer distribution of the lipophilic cations $MTPP^+$ or TPP^+ (^{3}H or ^{14}C as specified) as described pre-

viously [8]. Briefly, 1 μCi of the probe was added to the LUV system (1 mM phospholipid) which was incubated at room temperature for 1 h. An aliquot (0.1 ml) was withdrawn and the untrapped probe was removed by passing over a small (1 ml) Sephadex G-50 column. The trapped probe was determined by liquid scintillation counting employing a Packard 2000CA liquid scintillation counter and the phospholipid was determined by either phosphate assay or radiolabelled lipid ([^{3}H]DPPC) as specified. Binding of the lipophilic probe to the LUVs was determined for control preparations which were produced in either a K^+ or Na^+ buffer. Subsequently, these samples were diluted to appropriate lipid concentrations with the buffer in which they were prepared. Given the trapped volume determined employing [^{14}C]inulin the internal and external concentration of probe were obtained after correction for background binding [6] and the membrane potential was calculated via the Nernst equation. Transbilayer pH gradients were determined in a similar manner employing the weak base methylamine (^{14}C-labelled) as a probe. Methylamine was added to a final concentration of 1 μM containing 1 μCi/ml of [^{14}C]methylamine. Transmembrane pH gradients were calculated according to the equation: $\Delta pH = \log [MeAm]_i/[MeAM]_o$. Background association of methylamine with control LUVs was determined as described above.

The amount of dopamine accumulated was quantitated by adding dopamine from a 2 mM stock solution (containing [^{3}H]dopamine) to a given preparation to achieve 200 μM dopamine (1 μCi/ml labeled amine) and a 1 mM lipid concentration in a total volume of 1 ml. At appropriate time intervals, the unsequestered amine was removed by passing aliquots (0.1 ml) of the LUVs through 1 ml Sephadex G-50 columns. Dopamine and phospholipid phosphorus in the effluent were quantified by liquid scintillation analysis of [^{3}H]dopamine and phosphate assay. Where ATP was used as a trap, [^{14}C]cholesteryl oleate was incorporated in the original lipid film (1 μCi/20 μmol lipid) to quantitate lipid.

The uptake of epinephrine and serotonin was determined employing similar procedures as for dopamine. Accumulation of epinephrine was monitored using [^{3}H]epinephrine in systems containing 200 μM of the amine and 1 mM EPC LUVs. In the case of serotonin, similar concentrations of amine and lipid were utilized and the LUV-associated serotonin was assayed fluorometrically (excitation, 309 nm; emission, 340 nm) employing a Perkin-Elmer 650-10S fluorescent spectrophotometer after disruption of the vesicles with 0.5% Triton X-100.

Results

It has previously been briefly reported that dopamine and other biogenic amines can be accumulated inside lipid vesicles in response to a transmembrane pH gradient [3]. This is confirmed in Fig. 1 for the vesicle systems employed here, which consisted of egg PC LUVs with an established pH gradient (pH 4.6 inside, pH 7.5 outside). Incubation of these vesicles with dopamine (0.2 mM) results in amine accumulation to levels corresponding to 40 nmol/μmol phos-

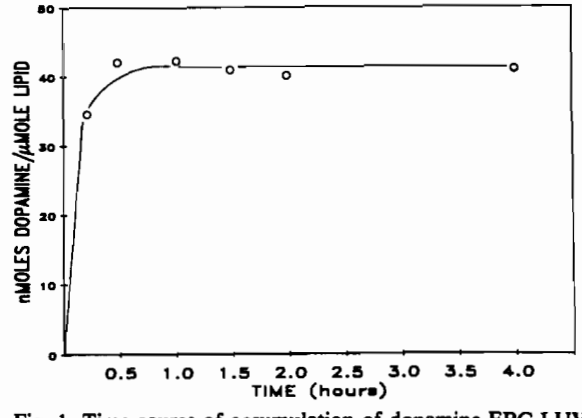

Fig. 1. Time course of accumulation of dopamine EPC LUV systems experiencing a transmembrane pH gradient (interior acid). The LUVs were prepared in a low pH buffer (pH 4.6) and the untrapped buffer exchanged for an iso-osmotic buffer at pH 7.5 (see Methods). Subsequently, the LUVs were diluted to achieve a 1 mM phospholipid concentration and a final dopamine concentration of 200 μM (1 μCi/ml [^{3}H]dopamine). Aliquots (0.1 ml) were removed at indicated time intervals, untrapped dopamine removed by gel filtration and the remaining dopamine assayed by scintillation counting. Uptake in control samples pH 4.6 in/pH 4.6 out or pH 7.5 in/pH 7.5 out resulted in no measurable association of dopamine.

pholipid. Given the measured trapped volume (1.5 μl/μmol lipid), this corresponds to an internal dopamine concentration of 25 mM and transmembrane concentration gradients of 160 (interior vs. exterior). Such transport is presumed to occur via the neutral (deprotonated) form of dopamine, resulting in redistributions according to the Henderson-Hasselbach equation such that

$$[AH^+]_{interior}/[AH^+]_{exterior} = [H^+]_{interior}/[H^+]_{exterior}$$

where AH^+ indicates the protonated form of the biogenic amine. For the concentration gradient of 160 observed here, such a distribution would correspond to ΔpH of 2.2. This is in close agreement with the measured (employing the pH probe methylamine) ΔpH of approximately 2.5. This relationship is defined more precisely by the data shown in Fig. 2. Vesicles were prepared with varying pH gradients (as described in the Methods) and subsequently dopamine uptake and ΔpH (employing methylamine) were determined. As indicated in the insert there is good correlation between the measured ΔpH and the interior/exterior distribution of dopamine.

These results clearly demonstrate that dopamine can be accumulated in LUV systems exhibiting a transmembrane pH gradient. This transport process would appear to be mediated by movement of the neutral species. It should be noted, however, that at pH 7.5 the predominant species present is the protonated form (pK_a for the amino group on dopamine is approximately 9.9). In previous work we have demonstrated that a variety of amino-containing drugs (chlorpromazine [6], dibucaine [9], and the antineoplastic agents, adriamycin and vinblastine [10]) can be accumualted into LUV systems in response to a K^+ diffusion potential (negative inside). This data appeared to suggest that the amino-containing weak bases could be accumulated in a positively charged (protonated) form in direct response to Δψ (negative inside). The studies presented in Fig. 3 were aimed at determining whether dopamine could be accumulated in such a manner. Addition of valinomycin to egg PC LUVs which exhibit Na^+/K^+ chemical gradients (K^+ inside) results in membrane potentials in excess of 100 mV (inside negative) as assayed by $MTPP^+$ [8]. As Fig. 3 indicates

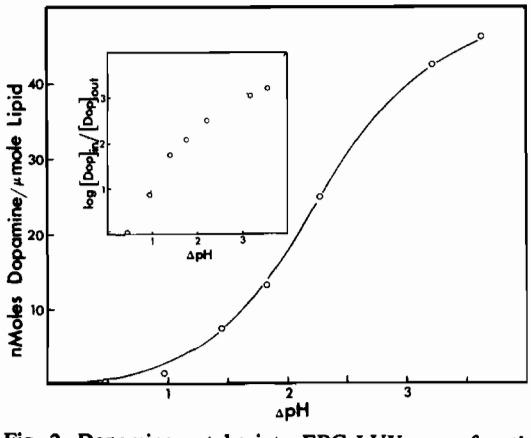

Fig. 2. Dopamine uptake into EPC LUVs as a function of the applied transmembrane ΔpH. LUVs were prepared at varying pH (ranging from 4.0—7.5) and subsequently the untrapped buffer was exchanged for buffer at pH 7.5 (see Methods). Conditions and methods for quantitating dopamine and ΔpH are specified in the legend of Fig. 1 and Methods.

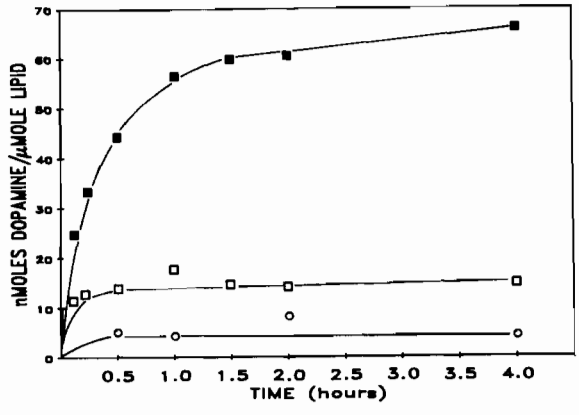

Fig. 3. Time course of the uptake of dopamine into EPC LUVs in response to a K^+ diffusion potential (Δψ). The LUVs were prepared in a potassium glutamate (KGlu) buffer and the untrapped buffer exchanged for an iso-osmotic NaCl buffer (see Methods). Conditions and methods for quantitating dopamine uptake were as indicated in the legend of Fig. 1 and Methods. The various symbols indicate: (■), uptake in the presence of an Na^+/K^+ chemical gradient and valinomycin or absence of valinomycin (□); (○), uptake in control samples containing valinomycin (0.5 μg/μmol phospholipid) with equal concentrations of K^+ on both sides of the membrane.

incubation of these LUVs with 0.2 mM dopamine results in the uptake of dopamine at a somewhat slower rate than observed for ΔpH uptake (Fig. 1). Levels in excess of 60 nmol/μmol phospholipid were obtained, corresponding to an internal dopamine concentration of 40 mM and transmembrane concentration gradient of 285. Little or no uptake is observed in control vesicles not exhibiting a K^+ diffusion potential.

It has previously been observed for sonicated SUV systems [11] that establishing a valinomycin-induced K^+ diffusion potential leads to the formation of a pH gradient (acidic inside). Redelmeier et al. [12] have shown for the LUV systems employed here that, in the absence of a permeant weak base, K^+ diffusion potentials can give rise to a small ΔpH (0.7 units). According to the data presented in Fig. 2, a ΔpH of this magnitude should result in dopamine accumulating to levels of approximately 2 nmol/μmol phospholipid. In order to obtain the dopamine gradients of 285 observed in Fig. 3, a ΔpH approaching 2.5 units would have to be established. To characterize the behaviour of the internal pH during dopamine accumulation, the pH probe methylamine was employed to monitor the intravesicular pH achieved on generation of Δψ. As shown in Fig. 4, the equilibrium ΔpH observed in the presence of dopamine is increased (ΔpH approx. 1.2) over the value in the absence of this amine (ΔpH approx. 0.7). The Δψ reaches a maximum value of 160 mV and subsequently decays to less than 100 mV (negative inside). This data indicates that upon accumulation of dopamine the Δψ and ΔpH approach electrochemical equilibrium. However, the equilibrium distribution of dopamine in response to Δψ would correspond to a pH gradient of 2.2 units which is significantly larger than the induced ΔpH observed (1.2 units).

In order to check this further, systems in which the induced ΔpH was varied by varying the buffering capacity of the intravesicular medium were constructed. As shown in Table I the induced ΔpH obtained at equilibrium in response to a valinomycin-induced ΔpH decreased from 1 pH unit for an interior buffer of 1 mM Hepes to 0.1 pH unit for 20 mM Hepes and 200 mM K_2HPO_4, whereas the Δψ remained constant at approximately 110 mV. Remarkably, the rate and extent of dopamine uptake in these systems are also relatively constant over this range of buffering capacities (Fig. 5). As was shown before in Fig. 4, the magnitude of the ΔpH at equilibrium increased in the presence of dopamine (Table I), whereas the Δψ values appeared to decrease with increasing interior buffering capacities. The decay of Δψ may be attributed to an efflux of K^+ ions to compensate for an inward flux of protons associated with dopamine accumulation.

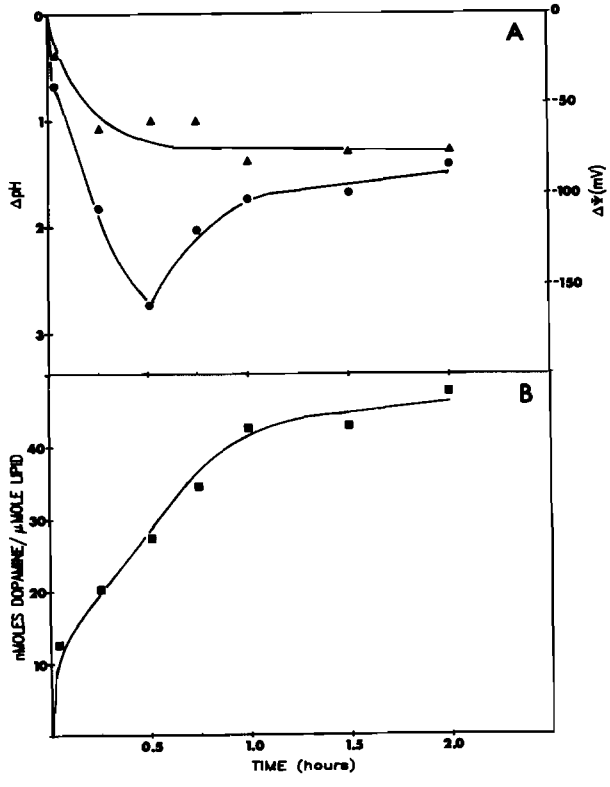

Fig. 4. Time course for the membrane potential (Δψ) (●) and transmembrane ΔpH (▲) developed in EPC LUVs prepared with a transmembrane Na^+/K^+ gradient following addition of dopamine and valinomycin (0.5 μg/μmol phospholipid). (A) Transmembrane pH gradients were measured employing [^{14}C]MeAm as described in Methods. The Δψ was measured employing [^{14}C]TPP as described in the legend of Fig. 3 and Methods. These estimates were made in the presence of 200 μM dopamine. (B) Utilizing the same preparation in A, dopamine uptake (■) was measured under the conditions described in Fig. 1.

TABLE I

Influence of increased buffering capacity on the measured equilibrium transmembrane pH and electrical gradients.

Buffer	Absence of dopamine		Presence of dopamine[c]		
	$\Delta\psi$[a]	ΔpH[b]	$\Delta\psi$	ΔpH	nmol dopamine / μmol lipid
1 mM Hepes	−112	1.0	−108	1.5	42.1
20 mM Hepes	−112	0.7	−106	1.3	39.8
20 mM Hepes*					
50 mM KP_i	−115	0.3	−98	0.9	38.7
125 mM KP_i	−112	0.3	−95	0.8	44.0
200 mM KP_i	−107	0.1	−90	0.7	37.7

[a]Membrane potentials were calculated from the transmembrane distribution of TPP^+ as described in Methods. Values were obtained after a 1-h incubation at room temperature.
[b]The pH gradient was measured employing methylamine as described in Methods after a 1-h incubation at room temperature.
[c]Dopamine was present at a concentration of 200 μM and uptake was determined as described in Methods after a 1-h incubation.

Given that the dopamine uptake in response to a K^+ diffusion potential is larger than can be accounted for by the induced pH gradients, questions arise as to the mechanism involved. It may be suggested that higher transmembrane gradients of dopamine may result from partitioning of the neutral form of dopamine into the vesicle membrane. The high lipid concentration experienced by the entrapped drug could then lead to higher interior dopamine concentrations than expected on the basis of a Henderson-Hasselbach equilibrium. This would appear unlikely since LUVs with an imposed pH gradient of 1 (see Fig. 2), which is similar to the ΔpH observed in the presence of the K^+ diffusion potential, results in uptake levels of less than 4 nmol/μmol phospholipid, which is appreciably less than observed for the K^+ potential-driven uptake (>40 mol/μmol phospholipid). It is important to note that the 1-unit pH gradient is maintained during accumulation of dopamine.

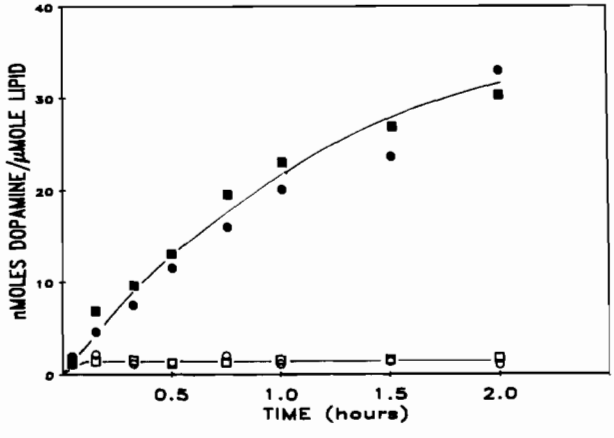

Fig. 5. Time course for the uptake of dopamine into EPC LUV systems with different (entrapped) buffering capacities. The LUVs were prepared in 125 mM K_2HPO_4 (●) or 125 mM K_2SO_4 (■) buffers containing 20 mM Hepes (pH 7.5). Untrapped buffer was exchanged for 125 mM Na_2SO_4, 20 mM Hepes (pH 7.5) and dopamine uptake monitored as indicated in Methods. The open symbols indicate uptake for control samples lacking a Na^+/K^+ gradient.

Another possible mechanism is that the charged (protonated) form is accumulated directly in response to $\Delta\psi$ by a K^+ anti-port mechanism similar to that observed for lipophilic cationic probes of membrane potential [6]. In order to evaluate this $\Delta\psi$-dependent mechanism uptake was measured at low pH values where the amino group of dopamine should be fully protonated. If $\Delta\psi$-dependent uptake is due to uptake of the protonated form, the uptake in response to $\Delta\psi$ should be maintained. As shown in Fig. 6 this is not the case. Dopamine accumulation is maximum in the region of

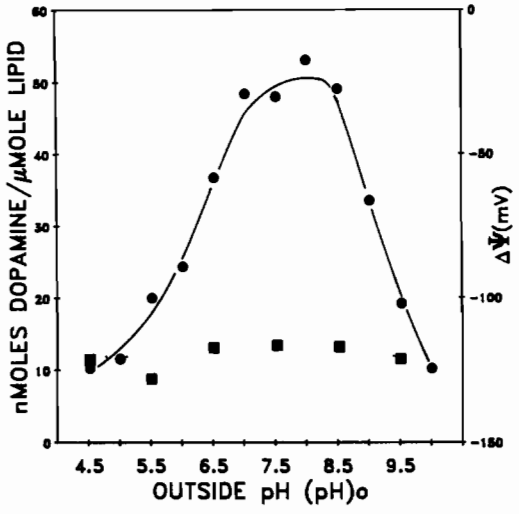

Fig. 6. (●), Influence of pH on dopamine uptake into EPC LUVs experiencing transmembrane Na^+/K^+ electrochemical gradients (K^+ inside) in the presence of valinomycin. The samples were prepared in the buffer systems indicated in Methods such that the interior and exterior pH was the same. Uptake was assayed as indicated in the legend to Fig. 1 and Methods following a 1-h incubation at 20°C. (■), Influence of pH on the membrane potentials observed for EPC LUVs experiencing Na^+/K^+ transmembrane chemical gradients (K^+ inside) in the presence of valinomycin. The potentials ($\Delta\psi$) were obtained by assaying the transmembrane distribution of MTPP (1 μCi [^{3}H]MTPP/ml) as indicated in Methods following a 1-h incubation at 20°C. The buffers employed and other experimental conditions were the same as above, with the exception that the EPC LUVs were not freeze-thawed prior to extrusion.

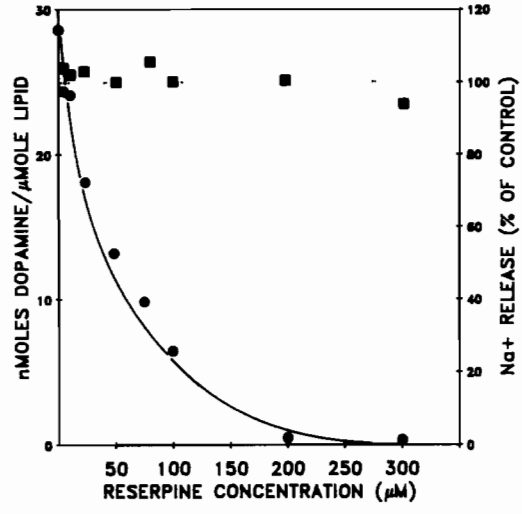

Fig. 7. (●), Influence of reserpine on uptake of dopamine into EPC LUVs prepared with a transbilayer pH gradient (inside acidic). LUVs were prepared in the K-Glu buffer (pH 4.6) and untrapped buffer was exchanged for a K-Glu buffer containing 20 mM Hepes (pH 7.5). Dopamine uptake was assayed as indicated in the legend to Fig. 1 and Methods following a 1-h incubation in the presence of 200 μM dopamine (1 μCi [^{3}H]dopamine/ml). The closed squares (■) indicate the retention of entrapped ^{22}Na (refer to axis label on right-hand side).

pH 7.5—8.0 and is much reduced at low pH values (pH 4.5). The valinomycin-induced K^+ diffusion potentials remain constant over the entire pH range.

Additional studies were performed in order to compare the uptake of dopamine into LUVs to the biological situation encountered in secretory granules such as the chromaffin granule. The first of these concerns the observation [15] that reserpine inhibits biological amine uptake. We therefore examine the influence of reserpine on dopamine accumulation into the LUV systems employed here (ΔpH 2.9). As shown in Fig. 7, 50 μM reserpine reduces the equilibrium uptake level of dopamine by approximately 50%. This concentration of reserpine, corresponding to 60 nmol/μmol lipid, is significantly higher than the concentrations required to inhibit epinephrine uptake (due to ΔpH or $\Delta\psi$) into chromaffin granule ghosts (0.2 nmol/μmol lipid [14]). It has been suggested that such reserpine levels result in a significant membrane permeabilization [14], but no significant leakage of entrapped ^{22}Na from the vesicles was observed even at the highest reserpine concentrations employed (375 nmol/mg lipid).

The second topic concerns the absolute levels of accumulated dopamine which can be achieved in response to K^+ or H^+ gradients, which fall in the range 30—40 mM. These are lower levels than observed (for total catecholamines) in chromaffin granules (approx. 0.5 M) and it has been suggested [13] that the high levels of entrapped ATP in the biological vesicle act as a sink for accumulated catecholamines. This was tested by trapping 125 mM

ATP inside LUVs and monitoring the ΔpH-dependent uptake. As shown in Fig. 8, the presence of internal ATP results in a marked enhancement in dopamine accumulation from 40 nmol/μmol lipid to 160 nmol/μmol lipid. This corresponds to dopamine concentration gradients of ⩾2500 (interior/exterior) which would suggest a ΔpH of 3.4 units. This gradient is 2.5 times greater than that predicted on the basis of the imposed ΔpH (approx. 3.0 units) in these LUV systems and could be consistent with formation of osmotically-insensitive dopamine-ATP complexes in the vesicle interior. However, in control preparations (pH_o = pH_i) the trapped ATP did not result in net accumulation of dopamine at either pH 4.5 or 7.5, suggesting that the interaction of dopamine with ATP is not enough to drive the uptake process. Interestingly, as shown in Fig. 9, LUVs with entrapped citrate (300 mM) at pH 4.0 accumulate dopamine to levels in excess of 180 nmol/μmol phospholipid. Again the concentration gradients achieved (6000 interior/exterior, corresponding to a ΔpH of 3.8 units) exceeds that predicted for the measured ΔpH of approximately 3 units.

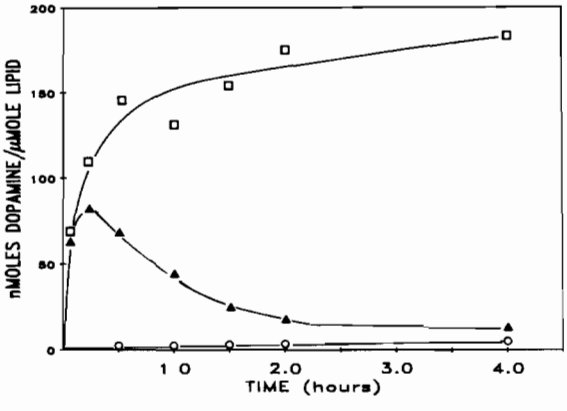

Fig. 8. Influence of trapped ATP on dopamine accumulation by EPC LUVs. LUVETs were prepared as described in Methods such that 125 mM ATP (pH 4.3 K^+ buffer) was trapped within the vesicles and a Na^+ buffer was exterior. The conditions for dopamine uptake were as described in the legend to Fig. 1. The open circles represent uptake by control samples, which have trapped ATP but no pH gradient (pH inside = pH outside). The other symbols represent uptake in the absence of added ionophores (□), or in the presence of both CCCP and valinomycin (▲) at concentrations described in the legend to Fig. 1. The dashed line represents uptake by vesicles with a transmembrane pH gadient and no trapped ATP (see Fig. 4).

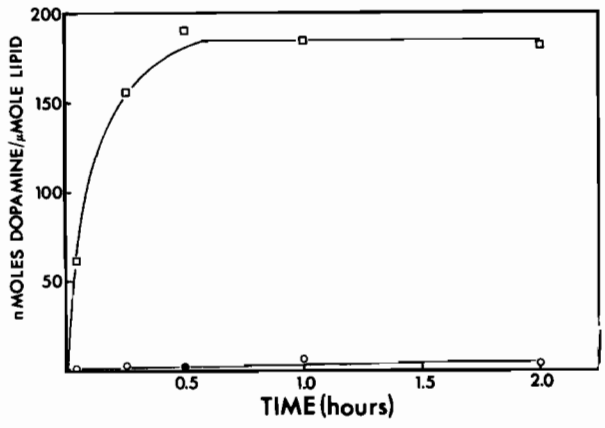

Fig. 9. Influence of trapped citrate on dopamine accumulation by EPC LUVs. Samples were prepared in the presence of 300 mM citrate (pH 4.0) and subsequently the untrapped buffer was exchanged for a Na^+ buffer (pH 7.5) as described in the Methods. Dopamine uptake was quantified as described in the legend to Fig. 1. The open circles represent uptake in control samples (pH inside = pH outside) which were maintained at pH 4.0 and pH 7.5 respectively. Open squares represent uptake in response to ΔpH.

A final series of experiments were performed to determine whether other biogenic amines could also be accumulated in response to a K^+ diffusion potential or pH gradient. As shown in Fig. 10, both epinephrine and serotonin can be accumulated in response to transmembrane proton and potassium ion gradients although the uptake levels obtained are significantly lower than for dopamine.

Discussion

The results of this investigation clearly show that a K^+ diffusion potential (negative inside) or a pH gradient (acidic inside) can cause uptake of dopamine and other biogenic amines into LUV systems. Such uptake can be significantly increased by the presence of entrapped anions such as ATP and citrate. Two important aspects of these results concern the mechanism whereby dopamine is accumulated in response to Δψ and possible relations to uptake of biogenic amines in vivo. We discuss these points in turn.

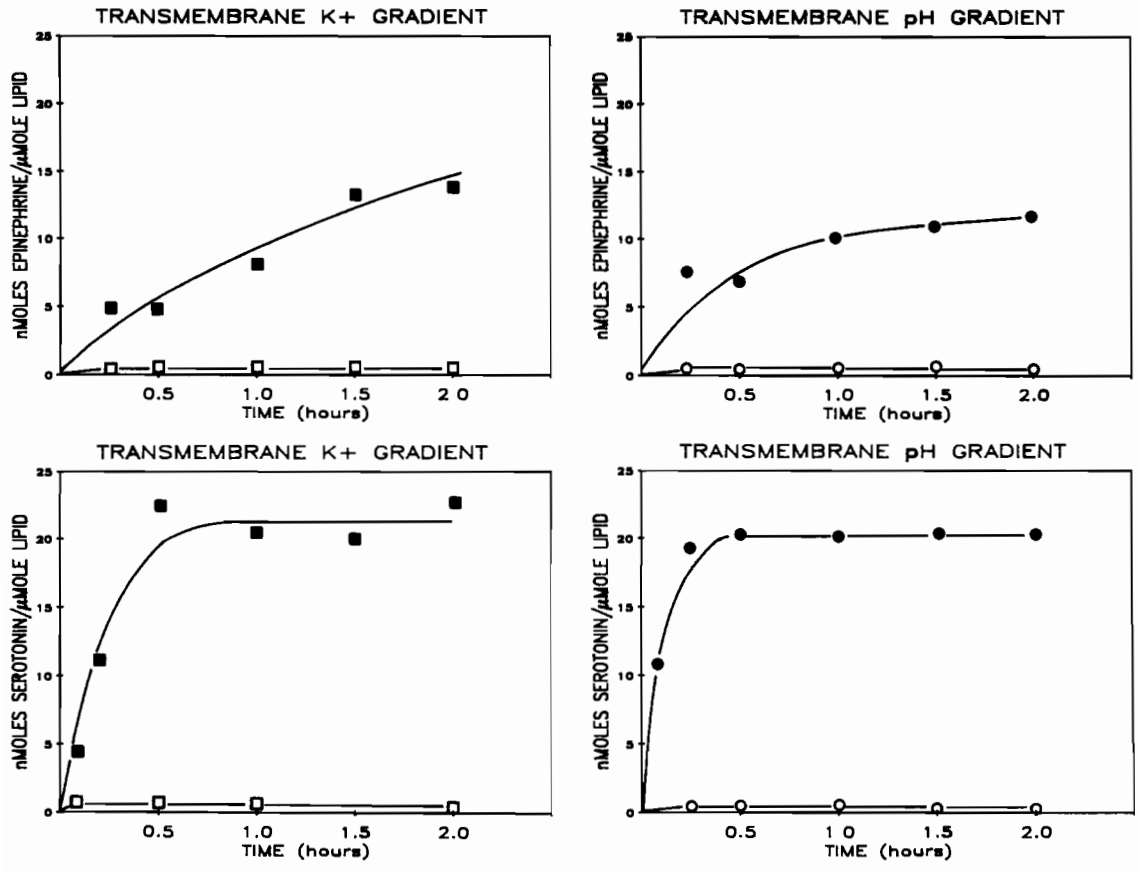

Fig. 10. Time course for the accumulation of epinephrine and serotonin by EPC LUVET systems with either a Na^+/K^+ ion gradient or a transmembrane pH gradient. Gradients were formed as described in Fig. 1. Open symbols represent uptake in control samples. The other symbols represent uptake in the presence of valinomycin (■) and in the absence of added ionophores (●).

The mechanism whereby dopamine is accumulated into LUVs in response to a K^+ diffusion potential (interior negative) is likely to be similar to the mechanism of accumulation of amino-containing drugs such as representative local anaesthetics (dibucaine, chlorpromazine), anti-cancer agents (adriamycin, vinblastine) and β-blockers (propranolol) noted elsewhere [6,9,10]. Our interpretation of this previous work has been largely guided by the similarity between the uptake of these amino-containing compounds and the uptake of compounds commonly employed as indicators of membrane potential (safranine, $MTPP^+$) which are lipophilic cations with delocalized positive charges [6]. This similarity has led to the suggestion that the amino-containing weak bases are accumulated in a positively charged (protonated) form in direct response to $\Delta\psi$ by an anti-port mechanism involving exchange for trapped K^+ ions. In particular, previous work from our laboratory indicated that dibucaine was actively transported in response to a K^+ diffusion potential [9]. It was suggested that dibucaine accumulated until the surface potential due to the internalized dibucaine approached a value which was equal and opposite to the K^+ diffusion potential. These arguments did not take into consideration the $\Delta\psi$-induced transmembrane pH gradients. Results presented here and elsewhere [11,12] clearly indicate that formation of the K^+ diffusion potential induces an influx of protons

resulting in a ΔpH (interior acidic) (Table I). Further, an increase in ΔpH is associated with dopamine accumulation. These data suggest that the accumulation process may be dependent on the redistribution of the neutral form of dopamine. As shown in Fig. 2, dopamine can redistribute across lipid bilayers according to the Henderson-Hasselbach equation which indicates that the neutral form of dopamine be permeable and the charged form impermeable.

The major difficulty in ascribing the $\Delta\psi$-dependent uptake of dopamine as due to a redistribution of the neutral species is that the equilibrium uptake level (approx. 40 nmol dopamine/μmol phospholipid corresponding to a log interior/exterior ratio of 2.2) far exceeds that predicted from the magnitude of the $\Delta\psi$-induced ΔpH (1.2). As indicated in the Results this effect cannot simply be attributed to a high membrane partition coefficient for dopamine. Vesicle systems exhibiting a ΔpH of 1.2 units give rise to dopamine uptake levels (< 5 nmol/μmol phospholipid) which are less than systems exhibiting a $\Delta\psi$-induced ΔpH of the same magnitude. An insight into the uptake mechanism can be obtained by examining the influence of entrapped ATP on net uptake levels (Fig. 8). It has been suggested that in chromaffin granules the high levels of entrapped ATP can act as a sink for accumulated catecholamines by forming an osmotically insensitive complex [13]. As shown here, the level of dopamine uptake in model LUV systems with entrapped ATP or citrate (Figs. 8 and 9 respectively) are significantly greater (2- to 6-fold) than would be predicted on the basis of the measured ΔpH. The high dopamine uptake levels obtained in response to $\Delta\psi$ may correspond to a non-equilibrium trapping phenomenon that may occur with specific amines. The resulting transmembrane distribution, therefore, could not be accounted for on the basis of a simple Henderson-Hasselbach equilibrium.

The relation between the results presented here and the accumulation of dopamine and other biogenic amines into secretory granules in vivo is of interest. Uptake of dopamine into chromaffin granules and derived "ghosts" can be driven by an inwardly directed proton pump [5] giving rise to a positive membrane potential as well as a ΔpH (inside acidic). In the absence of ATP, the ΔpH and the dopamine concentration gradient are maintained although the membrane potential decreases from + 50 mV to − 70 mV [4]. This indicates a primary role for ΔpH in maintaining dopamine gradients in vivo. In this regard it is interesting to compare the initial influx rates observed for dopamine uptake into LUV's in response to a pH gradient potential (Fig. 4) with those observed in granule ghosts on addition of ATP. Previous reports [16] indicate maximum dopamine uptake rates (V_{max}) of approximately 1.0 nmol/min/mg protein under conditions where dopamine is incubated with ghosts (0.9 mg protein in 1 ml). Assuming a lipid area per molecule of 0.6 nm^2 and a lipid-to-protein ratio of 2 (w/w) [18] for ghosts, this corresponds to dopamine uptake rates of 0.22×10^{-3} nmol/min per cm^2 at 25 °C. Under the conditions employed here (0.2 mM dopamine; 1 mM phospholipid, 20 °C) initial velocities for dopamine uptake of $\geq 1.7 \times 10^{-3}$ nmol/min per cm^2 are obtained, which are a factor of 7.5 faster. Although the different systems and conditions preclude any definitive conclusions, it would appear that the dopamine uptake rates observed in the model LUV's in response to established ion gradient can be of the same order or faster than those observed in chromaffin granule membranes.

In summary, the results presented here show that dopamine and other biogenic amines can be directly accumulated into LUV systems in response to a K^+ diffusion potential (inside negative) as well as a pH gradient (inside acidic). It is likely that at least part of the $\Delta\psi$-dependent accumulation can be attributed to the ΔpH generated on establishing the K^+ diffusion potential. The pH gradients measured are, however, too small to account for the dopamine concentration gradients achieved, suggesting a more complex mechanism of uptake. Finally, the rate of dopamine uptake in response to established ion gradients and the influence of entrapped ATP on net uptake levels are comparable with catecholamine uptake into chromaffin granules.

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