ELECTROGENIC TRANSPORT OF BIOGENIC AMINES IN CHROMAFFIN GRANULE MEMBRANE VESICLES

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Received 27 December 1979

1. Introduction

The catecholamine-rich, chromaffin-rich chromaffin granules are capable of accumulating these amines by a process requiring ATP [1,2]. This process is inhibited by reserpine [1], a competitive and reversible inhibitor of the catecholamine transporter [3], and also by proton ionophores [4]. Moreover, it has been shown that the granules contain an ATPase capable of generating a electrochemical proton gradient ($\Delta\tilde{\mu}_{H^+}$) consisting of a pH gradient (ΔpH), acid inside [5–10], and a membrane potential ($\Delta\psi$), interior positive [7,11,12]. These observations strongly suggest that the ATP-dependent accumulation is a consequence of two discrete steps:

(i) ATP-dependent generation of an electrochemical proton gradient;
(ii) Catecholamine uptake driven by this gradient or one of its components (ΔpH or $\Delta\psi$).

In fact with intact chromaffin granules some correlations have been found between the magnitude of $\Delta\psi$ [13,14] and ΔpH [14] and the levels of biogenic amines taken up. However an obvious drawback of the intact granules is that they contain high levels of endogenous amines, complexed with ATP and Mg^{2+} [15–17]. Studies with chromaffin granule ghosts, which makes it possible to study the transport process itself, have provided direct evidence for the involvement of ΔpH as a driving force for biogenic amine transport. Thus, when pH gradients (interior acid) are artificially imposed across the ghost membrane, this results in a reserpine-sensitive biogenic amine uptake [18–20]. The ability of $\Delta\psi$ to serve as a driving force, in addition to ΔpH, is not clear [18,19,21,22] and the concept has in fact been challenged [20]. However, artificial imposition of $\Delta\psi$ (interior positive) causes a small increase in the steady state accumulation of adrenaline [23]. Similar attempts of our own using 5-HT, which is transported by the same carrier [3], showed only marginal stimulations if any. Therefore we defined conditions in which the effect of artificially imposed membrane potential, if any, would be easier to demonstrate.

Here, we provide direct evidence for this possibility, since it is shown that when an artificial ΔpH is imposed, the accumulation of biogenic amines is affected by the polarity of $\Delta\psi$.

Abbreviations: 5-HT, 5-hydroxy tryptamine; $\Delta\tilde{\mu}_{BA} = \frac{2.3RT}{F} \log \frac{[BA]_{in}}{[BA]_{out}}$; BA, biogenic amine

2. Materials and methods

2.1. *Chemicals*

5-Hydroxy [G-^{3}H]tryptamine (26.2 Ci/mmol) and D,L-[7-^{3}H]noradrenaline hydrochloride (15.2 Ci/mmol) were purchased from New England Nuclear. Valinomycin was acquired from Sigma.

2.2. *Preparations*

Chromaffin granule membrane vesicles were isolated as in [19], except that when Na-loaded vesicles were used K-Hepes was replaced with Na-Hepes throughout the preparation. Moreover the Na-loaded vesicles resuspended in 0.3 M sucrose, 10 mM Na Hepes (pH 7.5) were centrifuged once more and resuspended in a minimal volume at 20–25 mg protein/ml before storage in liquid air. Reconstitution in the presence of appropriate loading media was done as in [24].

2.3. *Transport assays*

Membrane vesicles were thawed and loaded with either Na- or K-Hepes at pH 6.5 as in [19]. Aliquots (4 μl) of these vesicles were then diluted into 200 μl assay medium containing 100 mM sucrose, the radioactive biogenic amine and either 95 mM Na- or K-Hepes (pH 8.5) as indicated in the figure legends. Valinomycin (2.5 μM) was added when indicated. Reactions were terminated and radioactivity was assayed as in [19] except that 0.3 M sucrose containing 10 mM K-Hepes (pH 8.5) was used as a stop solution.

2.4. *Protein determinations*

These were performed as in [25].

3. Results

The imposition of ΔpH by dilution of chromaffin granule ghosts loaded with sodium containing buffer at pH 6.5 into a potassium containing assay medium buffered at pH 8.5 results in a fast accumulation of 5-HT which reaches a steady state value of ~23 pmol/mg protein (fig.1A) after 1 min. Using the value of 3.9 μl/mg protein for the intravesicular volume [19] a concentration gradient of ~65 is reached. When the dilution medium contains valinomycin, a condition expected to superimpose a membrane potential (interior positive) on top of the ΔpH, a pronounced increase of both initial rate and extent of 5-HT accumulation is observed and reserpine, a competitive and reversible inhibitor of the biogenic amine carrier, strongly inhibits the process (fig.1A). Since artificially imposed membrane potentials across this membrane decay rather quickly [23] the stimulation of accumulation is transient. Nevertheless, the concentration gradient rises from 65–137, and in the experiment depicted in fig.1B an even higher gradient (228-fold) is observed. It can be seen that in the latter experiment valinomycin stimulates 5-HT accumulation only under conditions in which $\Delta\psi$ is generated ($[K^+]_{out} > [K^+]_{in}$).

It is also of interest to note that although valinomycin stimulates the accumulation of 5-HT, the decay of this accumulation is accelerated by the ionophore. In fact at later times the level of accumulation in the presence of the ionophore is lower than in its absence. Thus it seems that the co-ion permeability, in this case potassium, limits the decay of ΔpH. It

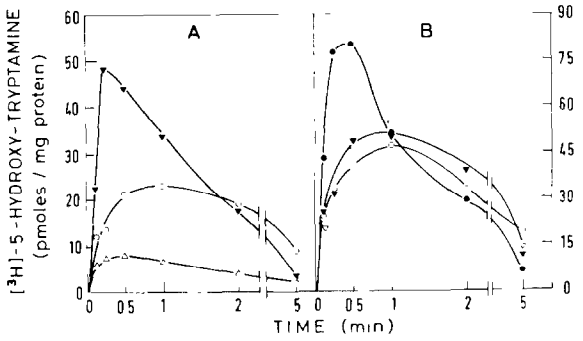

Fig.1. Effects of valinomycin and reserpine on ΔpH-driven 5-HT transport with superimposed potassium ion gradient (out > in). (A) Sodium-loaded vesicles (101 μg protein/assay) and the potassium containing assay medium supplemented with 0.09 μM 5-[^{3}H]HT (0.5 μCi/assay) were used. The following additions were made: (○–○) none; (▼–▼) valinomycin, 2.5 μM (△–△) reserpine, 2 μM in the latter case an aliquot of the vesicles was preincubated on ice with reserpine at the same concentration present in the assay medium. (B) Sodium loaded vesicles (64 μg protein/assay) and the potassium (circles) or sodium (triangles) containing assay media were used, both containing 0.09 μM 5-[^{3}H]HT (0.5 μCi/assay). The experiments were done in the absence (open symbols) or presence (closed symbols) of valinomycin, 2.5 μM. Since both valinomycin and reserpine stock solutions were in DMSO controls containing identical amounts of DMSO (1–1.5%) were run and the results were identical to those in the absence of DMSO.

should be stressed that clear and highly reproducible stimulations by valinomycin are only observed when a ΔpH of 2 units (acid interior) is imposed. Thus with a ΔpH of 1 unit it was much more difficult to observe the effect and with no ΔpH hardly any effect was seen (data not shown). Results similar to those with 5-HT are also observed with norepinephrine (fig.2). This is to be expected since we have recently shown that all biogenic amines are translocated by one carrier [3].

This carrier was recently solubilized and reconstituted in our laboratory [24]. Results similar to those described above are also obtained using the reconstituted system (data not shown).

Further support for the contention that $\Delta\psi$ (interior positive) serves as a driving force for biogenic amine accumulation is derived from the experiment depicted in fig.3. Potassium-loaded vesicles buffered at pH 6.5 are diluted into sodium containing media at pH 8.5. The addition of valinomycin is expected to cause a membrane potential (interior negative) with a polarity opposite to that under the experimental con-

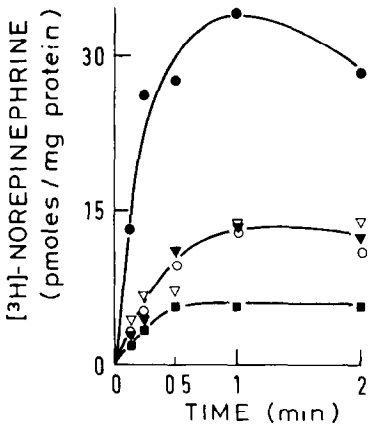

Fig.2. Effects of valinomycin and reserpine on ΔpH-driven norepinephrine transport with superimposed potassium ion gradient (out > in). Sodium loaded vesicles (96 μg protein/assay) and the potassium (circles) or sodium (triangles) containing assay media supplemented with 0.16 μM [^{3}H]noradrenaline (0.5 μCi/assay) were used. These experiments were done in the absence (open symbols) or presence (closed symbols) of 2.5 μM valinomycin. An experiment using the same vesicles preincubated with reserpine as above, with the potassium containing medium supplemented with 2.5 μM valinomycin and 2 μM reserpine is represented by the filled squares.

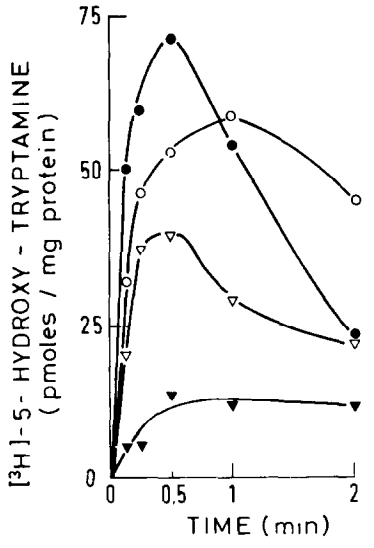

Fig.3. Effect of valinomycin on ΔpH driven 5-HT transport with superimposed potassium ion gradient (in > out). Potassium-loaded vesicles (28.4 μg protein/assay) and the potassium (circles) or sodium (triangles) containing assay media supplemented with 0.09 μM 5-[^{3}H]HT (0.5 μCi/assay) were used. The experiments were done in the absence (open symbols) or presence (closed symbols) of 2.5 μM valinomycin.

ditions of fig.1,2. It can be seen that under the conditions of this experiment the addition of the ionophore has opposite consequences for 5-HT accumulation, a strong inhibition is observed (fig.3). Again the effects are consistent with the well-known specificity of the ionophore. When the potassium loaded vesicles are diluted in potassium containing media, valinomycin does not inhibit. It actually stimulates 5-HT accumulation, presumably by charge compensation, allowing potassium entry in response to exit of positive charge caused by the translocation of 5-HT.

4. Discussion

The effect of an artificially imposed $\Delta\psi$ on ΔpH driven 5-HT and norepinephrine transport provides direct evidence that both components of the $\Delta\tilde{\mu}_{H^+}$ generated by the ATPase represent driving forces for biogenic amine transport. The ability of $\Delta\psi$ (interior positive) to stimulate in the presence of ΔpH (interior acid) is consistent with the polarity of both components generated by the granule membrane ATPase. As such the above results exclude the possibility of an electroneutral mechanism [18–20,22]. Instead it seems reasonable to postulate that the carrier acts as an antiporter translocating the biogenic amine in one direction and n protons in the opposite direction [14,23].

If the translocated species is the positive form $n + 1$ protons would have to be moved in the opposite direction. This means that with a given protonmotive force ($\Delta\tilde{\mu}_{H^+}$) higher concentration ratios of the biogenic amines ($\Delta\tilde{\mu}_{BA}$) can be achieved.
For $n = 1$:

$$\Delta\tilde{\mu}_{BA} = 2\left(2.3\frac{RT}{F}\log\frac{[H^+]_{in}}{[H^+]_{out}}\right) + \Delta\psi \qquad (1)$$

as opposed to:

$$\Delta\tilde{\mu}_{BA} = 2.3\frac{RT}{F}\log\frac{[H^+]_{in}}{[H^+]_{out}} \qquad (2)$$

for an electroneutral process. It is possible that the maximum $\Delta\tilde{\mu}_{BA}$ is actually never reached since in the absence of carrier biogenic amines can also distribute according to ΔpH [24,26]. There are some indications that with ATP-dependent transport values

of $\Delta\mu_{BA}$ higher than those dictated by (2) are observed. Thus a concentration gradient of epinephrine of 1100 is observed while under similar conditions the proton concentration gradient is only 170 [10,19]. Also in fig.1 concentration ratios higher than those dictated by ΔpH alone are observed notwithstanding the fast decay of $\Delta\psi$. Accumulation of biogenic amines according to (1) indicates that ΔpH is the dominant component of the driving force. In addition, the $\Delta\tilde{\mu}_{H^+}$ generated by ATP in the presence of chloride is mostly in the form of ΔpH [12]. This may provide an explanation for the observation that valinomycin (collapsing $\Delta\psi$) does not inhibit ATP-dependent accumulation and that nigericin (collapsing ΔpH) does inhibit strongly [18,19]. The higher concentration ratios which may be achieved by an electrogenic mechanism also may explain the fact that reserpine inhibits very strongly even though 5-HT and other amines can equilibrate across the membrane in the presence of ΔpH [24,26].

Acknowledgements

We wish to thank Drs G. K. Radda and D. Njus for stimulating discussions. This research was supported by grants from the Israel Center for Psychobiology and The Joint Research Fund of the Hebrew University and Hadassah.

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