Biochimica et Biophysica Acta 816 (1985) 403–410
Elsevier

BBA 72636

Transport of 5-hydroxytryptamine in membrane vesicles from rat basophilic leukemia cells *

Baruch I. Kanner and Annie Bendahan

Laboratory of Neurochemistry, Institute of Biochemistry, Hadassah Medical School, The Hebrew University, Jerusalem (Israel)

(Received December 13th, 1984)

Key words: Serotonin transport; Hydroxytryptamine transport; Membrane vesicle; Plasma membrane; Storage organelle; (Rat basophilic leukemia cell)

Rat basophilic leukemia (RBL) cells were grown as tumors. Membrane vesicles were isolated from them and serotonin transport was measured. Two types of transport were identified. One was sensitive to imipramine and sodium-dependent, while the other was sensitive to reserpine and ATP-dependent. The transport systems exhibit different affinities for serotonin (sodium-dependent, K_m 0.22 μM; ATP-dependent, K_m 2.6 μM) and are different in their substrate specificity, the former being much more specific. The 5-hydroxytryptamine transport by the reserpine-sensitive system was strongly inhibited by other biogenic amines, like norepinephrine, epinephrine and dopamine, whereas that by the imipramine-sensitive system was not. Upon Ficoll gradient centrifugation the two transport systems were separated. The reserpine-sensitive activity was found much further into the gradient than the imipramine-sensitive one. The latter co-migrated with the receptor of IgE, which is located in the plasma membrane. Characterization of latter showed that in addition to the dependence of 5-hydroxytryptamine influx on external sodium it was also absolutely dependent on external chloride and was strongly stimulated by internal potassium. On the other hand, efflux required external potassium. An alternative potassium independent way of loss of labelled 5-hydroxytryptamine was by exchange. A small but consistent stimulation of influx was observed in the presence of valinomycin, indicating that the process is electrogenic. The reserpine-sensitive system could also be driven in the absence of ATP. This required the imposition of pH gradient (acid inside) and was stimulated by an artificially imposed membrane potential (positive inside).

Introduction

Two types of transport systems for biogenic amines have been identified in the nervous system [1,2] and in platelets [3–5]. The first, located in the plasma membrane, is sodium-dependent and sensitive to tricyclic antidepressants such as imipramine [1,3,6]. In the intact cells accumulation of the amine is powered by the $(Na^+ + K^+)$-ATPase, which creates a sodium gradient (out > in). The latter provides the direct driving force for the accumulation, which occurs via co-transport of the amines with sodium [6]. Once the biogenic amines enter the cell they are sequestered into storage organelles such as chromaffin granules (reviewed by Njus et al. [7]), synaptic vesicles [8,9] and plaelet storage organelles (see, for example, Ref. 10), respectively. The uptake into these organelles

* The first stages of this work were performed during a sabbatical visit (of B.I.K.) at the laboratory of Dr. H. Metzger, ARB, NIADDK, National Institutes of Health.

Abbreviations: RBL, rat basophilic leukemia; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone.

occurs via a different transport system which is ATP-dependent and reserpine-sensitive. The organelles contain a H^+-ATPase which pumps protons into the organelles. Amine transport then occurs via antiport with a proton (reviewed in Ref. 7).

Also in mast cells histamine- and serotonin-containing storage organelles have been identified (see Ref. 11). In addition it has been shown that the organelles maintain an internal acid pH [12]. Furthermore they are able to exocytotic release of the amine (see Ref. 11). One way to measure such release in the related rat basophilic leukemia (RBL) cells is to preincubate the cells with radioactive serotonin and then to subsequently provoke exocytotic release [13,14]. The latter observation suggests that the cell line should also have a serotonin transport system located in the plasma membrane in addition to one located in storage organelles.

The aim of the present study was to identify such transport systems from the RBL cell line and to study their relationship with the known systems from other sources.

Materials and Methods

Materials

5-Hydroxy[^{3}H]tryptamine was from New England Nuclear or Amersham International. Nigericin was a generous gift of Dr. R.J. Hosley from Eli Lilly. Imipramine, reserpine, valinomycin and CCCP were from Sigma. All other materials were of the highest purity commercially available.

Methods

Preparation of membrane vesicles. RBL cells were injected into 7–10-day-old rats at 10^7 cells per animal. After 7–8 days the animals were killed by exposure to ether. The tumors were removed from the peritoneal cavity. Subsequent steps were done at 0–4°C. They were homogenized at a density of 1.5–2 tumors per 40 ml 0.25 M sucrose/10 mM Tris-HCl (pH 7.4) by eight strokes in a glass-Teflon homogenizer. For the initial experiments the mixture was centrifuged for 5 min at $1000 \times g$. The supernatant was then spun for 15 min at $39\,000 \times g$. The pellet (TP_2) was resuspended in sucrose/Tris at 15–20 mg protein/ml, divided into small aliquots, frozen and stored in liquid air. In the later experiments the tumor extract was spun for 15 min at $12\,000 \times g$ and the supernatant for 70 min at $48\,000 \times g$. The pellet (TP_3) was aliquoted and frozen as above and stored in a Revco freezer at −70 to −80°C. Higher specific activities of 5-hydroxytryptamine transport were found in the TP_3 fraction, and for this reason this fraction was preferred. For the experiment depicted in Fig. 2, the TP_2 preparation was used as the starting material for the gradient fractionation. All the other experiments illustrated were done with TP_3 although they were also done with TP_2 and gave similar results.

Electron microscopy of the preparation revealed various kinds of membrane particles: (1) small (empty) membrane vesicles, diameters 0.1–0.3 μm; (2) large (empty) membrane vesicles, diameters 0.6–0.8 μm; (3) small membrane vesicles filled with cytoplasm, diameters 0.1–0.3 μm. Class 3 were the most abundant. In addition ribosomes could be observed but the preparation was devoid of mitochondria.

Transport assays. Quite a bit of variation in the absolute rates and extents of transport was observed from preparation to preparation. However, with the same preparation very similar values were obtained even when measured on different days. Representative experiments are shown.

Na^+-dependent transport. An aliquot of vesicles was rapidly thawed in a water bath at 37°C. Subsequently 20 volumes of the loading solution (usually 100 mM potassium phosphate at pH 6.8) were added and the mixture was incubated for 10 min at 37°C; this was followed by a centrifugation step ($27\,000 \times g$ for 15 min) and the pellet was resuspended in the loading solution at a protein concentration of 4–10 mg/ml. Approx. 40–100 μg of membrane protein (10 μl of volume) were diluted into 190 μl of 0.15 M NaCl (or other medium as indicated in the legends to the figures) containing 1 μCi of 5-hydroxy[^{3}H]tryptamine (9–17.3 Ci/mmol) at room temperature. After various times, reactions were stopped by addition of 2 ml ice-cold 0.15 M NaCl, filtered, washed and the filters were processed as described previously [15]. Dilution-induced efflux was measured after influx as follows: Membrane vesicles (5 μl) were added to 45 μl 0.15 M NaCl containing 1 μCi of 5-hydroxy[^{3}H]tryptamine; after 5 min of influx

the mixture was diluted with 1 ml of the appropriate efflux solution (see figure legends). At various times reactions were stopped and transported 5-hydroxy[^{3}H]tryptamine was determined as above.

ATP-dependent transport. This was determined essentially as described previously [16]. Membrane vesicles (10 μl, 150–200 μg of protein) were diluted into a medium containing 0.25 M sucrose/10 mM potassium Hepes (pH 8.5)/2.5 mM $MgCl_2$/5 mM disodium ATP (the same results were obtained when dipotassium ATP was used instead, data not shown) and 1 μCi of 5-hydroxy[^{3}H]tryptamine (9–17.3 Ci/mmol), at 37°C. At the indicated times the reaction was terminated by addition of 2 ml of ice-cold 0.25 M sucrose + 10 mM potassium Hepes (pH 8.5) filtration on nitrocellulose filters (Schleicher and Schull, 0.45 μm pore size) and washing with another 2 ml of the same solution. The filters were then dried and counted. Dilution-induced efflux was measured as described [17].

Serotonin transport driven by ΔpH and $\Delta\psi$ was measured as follows: Membrane vesicles were loaded at the desired pH (usually 6.5) and ionic composition by osmotic shock and subsequent resealing as described [16]. Transport was measured upon dilution of these vesicles into the desired 'out' medium containing 1 μCi 5-hydroxy[^{3}H]tryptamine with and without valinomycin. The composition of the 'loading' and 'out' media are given in the figure legends. Reactions were stopped and processed as above.

Labelling of IgE receptors. The cell homogenate was incubated with 0.25 μg/ml ^{125}I-labeled IgE and $2 \cdot 10^5$ cpm/ml. After 10 min the extract was fractionated and the receptor distribution was followed by counting aliquots of each fraction for ^{125}I in a gamma counter.

Results

Two uptake systems for 5-hydroxytryptamine

Upon incubation of the vesicles isolated from rat basophilic leukemia (RBL) cells, grown as tumors in rats, with 5-hydroxytryptamine at 37°C, the amine is taken up and this process is greatly enhanced by ATP (Fig. 1A). Like ATP-dependent amine transport from other sources, the process in the vesicles from RBL was inhibited by 1 μM reserpine (Fig. 1A) but not by the inhibitor imipramine (see below). Similar ATP-dependent uptake was found in vesicles prepared from RBL cells grown in tissue culture (data not shown). Uptake of histamine, which is also exocytotically released, is also ATP-dependent, but very poorly as compared to 5-hydroxytryptamine (data not shown).

The membrane preparation used in the experiment described in Fig. 1A is a crude preparation and is also expected to contain vesicles derived from other membranes, such as the plasma membrane. The plasma membrane from other sources such as brain or platelets contain a distinct uptake system for 5-hydroxytryptamine, which is sodium-dependent and sensitive to imipramine but not to reserpine. Since it was found that intact RBL cells have a sodium-dependent 5-hydroxytryptamine uptake system (data not shown), the vesicle preparation was tested for this activity (Fig. 1B). In this experiment the vesicles were preloaded with 0.1 M potassium phosphate (pH 6.8) and diluted into sodium chloride containing radioactive 5-hydroxytryptamine. Under these conditions, in the absence of any added ATP, uptake of 5-hydroxytryptamine is observed which is strongly inhibited by 2.5 μM imipramine, but not by re-

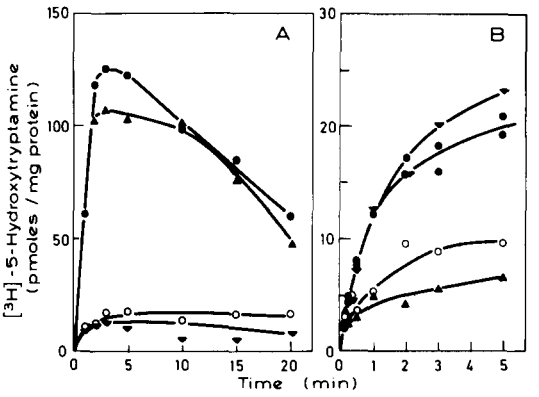

Fig. 1. The effect of imipramine and reserpine on 5-hydroxytryptamine transport. (A) ATP-dependent transport. This was measured as described under Materials and Methods using 160 μg membrane protein. (●) control; (▼) with 1 μM reserpine; (▲) with 2.5 μM imipramine; (○) minus ATP. (B) Sodium-dependent transport. In this experiment 95 μg of protein was used. (●) control; (▼) with 1 μM reserpine; (▲) with 2.5 μM imipramine; (○) with 5 μM nigericin.

serpine (Fig. 1B). The process is also inhibited by the ionophore nigericin (Fig. 1B) which under this condition is expected to collapse the sodium (and potassium) gradient by exchanging internal potassium for external sodium. We have attempted to measure sodium-dependent 5-hydroxytryptamine transport in vesicles derived from RBL cells grown in culture, using hypotonic conditions to get appreciable cell breakage. However, the resulting vesicles only displayed the ATP-dependent uptake activity. This is also the reason that the membrane from the tumors were isolated under isotonic conditions. In fact, subjecting the preparation, derived from tumors, to osmotic shock did abolish the sodium-dependent activity. The reasons for this are not yet understood.

The two 5-hydroxytryptamine uptake systems also differ in their substrate specificity. The ATP-dependent uptake of 5-hydroxytryptamine, which has a K_m of 2.6 μM and a V_{max} of 850 pmol/min per mg protein (data not shown), is strongly inhibited by various biogenic amines such as norepinephrine, epinephrine and dopamine (all at 20 μM) (Table I). This indicates that the system has a broad substrate specificity just like its counterpart from the chromaffin granules [18]. On the other hand, sodium dependent 5-hydroxytryptamine uptake is much less sensitive to those amines (Table I), indicating a much greater specificity. Its affinity for 5-hydroxytryptamine is higher than that of the ATP-dependent one; the K_m was found to be 0.22 μM and its V_{max} was 90 pmol/min per mg protein.

The two uptake systems are anticipated to be located on different membrane vesicles, namely those derived from the plasma membrane (Na^+-dependent uptake) and those from the storage organelles (ATP-dependent uptake), respectively. Evidence directly supporting this is presented in Fig. 2. Membrane vesicles were layered on a con-

TABLE I

EFFECT OF BIOGENIC AMINES ON THE INITIAL RATE OF 5-HYDROXYTRYPTAMINE TRANSPORT

ATP- and sodium-dependent 5-hydroxytryptamine transport were studied as described in Materials and Methods. The reactions were allowed to proceed for 1 min. Added amines were present at 20 μM final concentration. Transport is expressed as counts per min ($\pm$S.D.). The amounts of 5-hydroxytryptamine transported for the controls (at 1 min) were 162.7 and 37.1 pmol/mg protein for ATP-dependent and Na^+-dependent transport, respectively.

Additions	Transport rate (cpm)	
	ATP-dependent	Na^+-dependent
None	60000 ± 2100	7200 ± 300
Dopamine	9400 ± 400	7200 ± 300
Norepinephrine	13200 ± 1600	7000 ± 400
Epinephrine	15900 ± 900	7000 ± 400
5-Hydroxytryptamine	7000 ± 400	600 ± 300

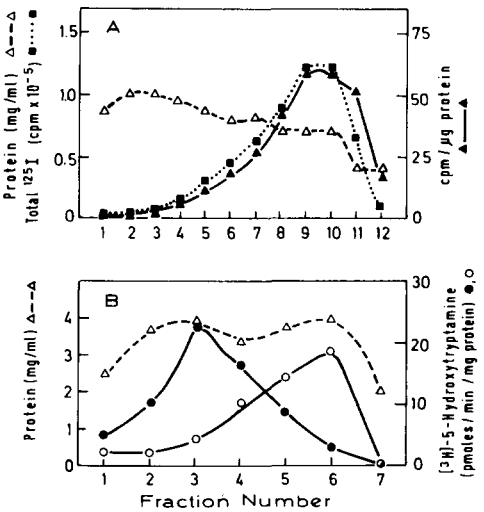

Fig. 2. Fractionation of the membrane preparation by Ficoll gradient centrifugation. The tumors of 8 rats were homogenized in 300 ml 0.25 M sucrose and 10 mM Tris-HCl (pH 7.4). 40 ml of this homogenate were set aside, trace labelled with ^{125}I-labeled IgE and fractionated as the remainder. 2 ml of the unlabelled TP_2 fraction was layered on a linear gradient of 5–25% Ficoll in 0.25 M sucrose and 10 mM Tris-HCl and centrifuged for 90 min in a SW.27 rotor. Seven fractions of 5 ml each were collected (Fraction 1 is the lowest, i.e., highest density) and each was assayed for protein (Δ), and upon pelleting for ATP-dependent (●) and sodium-dependent (○) 5-hydroxytryptamine transport. The latter was done upon preloading the vesicles with 0.1 M potassium phosphate (pH 6.8). This part of the experiment is depicted in the lower panel. In parallel the 0.85 ml (out of 0.9 ml) of the TP_2 obtained by the radioactive fractionation was centrifuged on an identical gradient. Eleven fractions of 3 ml (1 is the lowest) and a twelfth fraction of 1.5 ml were collected, assayed for protein (Δ) and for ^{125}I radioactivity (■). This is represented in the upper panel. The scale is drawn so that in both panels any distance plotted from the origin represents the same position on each of the gradients. The physical appearance of the gradients was: Two diffuse bands and a pellet. The pellet did not contain reserpine- or imipramine-sensitive 5-hydroxytryptamine transport, but did contain 63000 cpm of ^{125}I-labeled IgE. It probably represents broken membrane fragments.

tinuous (5–25%) Ficoll gradient and centrifuged in a SW 27.2 rotor for 2 h. Fractions were collected from the bottom and were assayed for both uptake activities. In addition a sample of vesicles from the same preparation, with the only difference that a portion of the original cell free extract had been trace labelled with IgE iodinated with ^{125}I to monitor the distribution of the IgE receptors, was run on a parallel gradient. It can be seen that the ATP-dependent activity moves towards the bottom and is well separated from the much lighter vesicles containing the sodium-dependent activity (Fig. 2, lower panel). The latter activity moves to the same density as the IgE receptors as judged by the ^{125}I counts (Fig. 2, upper panel). Since the IgE receptors are known to be located in the plasma membrane, it appears that as expected the sodium dependent activity originates from this membrane.

The Na^+-dependent 5-hydroxytryptamine uptake

As can be seen (Fig. 3) 5-hydroxytryptamine transport requires not only sodium but chloride as well. Furthermore, only in this case was the uptake inhibited by nigericin. Although in the figure the sodium and chloride were replaced by lithium and glucuronate, respectively, the same results were also obtained with other ions, such as choline and phosphate (data not shown). In the absence of

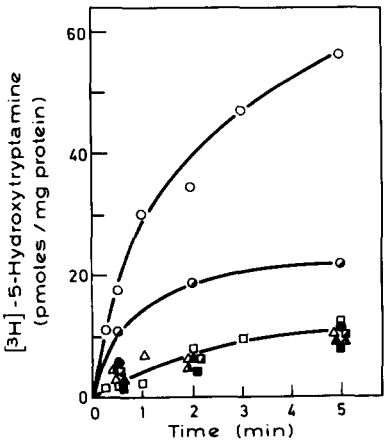

Fig. 3. The effect of external ions on imipramine-sensitive 5-hydroxyryptamine transport. Protein 64 μg per time point. Circles; 100 mM NaCl; triangles: 100 mM LiCl; squares: 100 mM sodium glucuronate. Open symbols: control; closed symbols: plus 2.5 μM imipramine; half closed symbols: plus 5 μM nigericin.

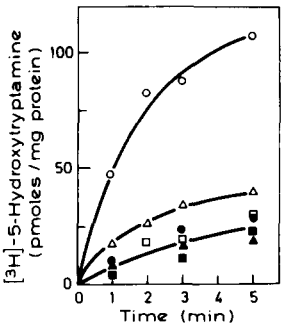

Fig. 4. The effect of internal cations on imipramine-sensitive 5-hydroxytryptamine transport. The internal medium conained 0.1 M potassium phosphate (pH 6.8) (circles, 75 μg protein); 0.1 M lithium phosphate (pH 6.8) (triangles, 60 μg protein) or 0.1 M Tris phosphate (pH 6.8) (squares, 55 μg protein). Open symbols: control; closed symbols: plus 2.5 μM imipramine.

either of these ions some uptake was observed but in this case it was not sensitive to imipramine, and not to nigericin, and probably reflects binding of 5-hydroxytryptamine to the membrane.

It is of interest to note that internal potassium is required for the uptake system as well. In its presence imipramine sensitive transport is detected, whereas when internal potassium is replaced by Tris or lithium, very little, if any, such uptake is detected (Fig. 4).

The process appears to be electrogenic. Addition of the potassium ionophore valinomycin is expected to create or enhance the magnitude of the membrane potential, interior negative, because of the potassium ion gradient (in > out). Stimulation of 5-hydroxytryptamine transport (up to 2-fold) was, however, not observed with all preparations. An experiment with an intermediate (about 40%) stimulation is depicted in Fig. 5. When this experiment was performed using an external medium lacking chloride the addition of valinomycin still did not promote any imipramine sensitive transport. This experiment precludes the possibility that the requirement for external chloride is due to charge compensation by this anion of electrogenic sodium-coupled 5-hydroxytryptamine influx.

Since sodium and chloride dependent influx of 5-hydroxytryptamine requires internal potassium, it was of interest to know whether efflux of the amine has a similar requirement, just like in the case of platelet vesicles [19]. Membrane vesicles were allowed to accumulate 5-hydroxytryptamine

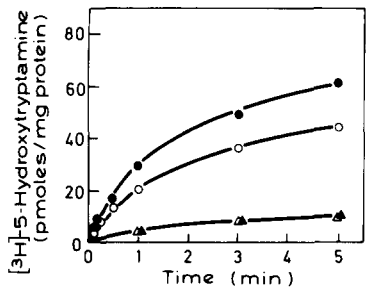

Fig. 5. The effect of valinomycin on sodium-dependent 5-hydroxytryptamine transport. 63 μg of the potassium-loaded vesicles were used. Open symbols: control containing 1 μl of DMSO; closed symbols: containng 2.5 μM valinomycin (added in 1 μl DMSO); circles: no further additions; triangles: 2.5 μM imipramine was also added.

in response to a sodium chloride gradient and subsequently 20-fold diluted in a variety of media. It can be seen that significant net afflux of 5-hydroxytryptamine occurs optimally when the dilution medium contains potassium (Fig. 6). Just as for the 5-hydroxytryptamine transport system in platelets, there exists an alternative way of exit for the labelled amine, namely exchange [19]. This is achieved by adding unlabelled 5-hydroxytryptamine to the efflux medium. In this case no external potassium is required (Fig. 6). The exchange is optimally observed in the presence of external sodium and chloride, indicating that the stimulation of efflux by external 5-hydroxytryptamine occurs via the transport system.

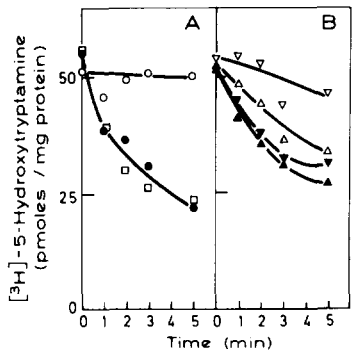

Fig. 6. Dilution induced efflux of 5-hydroxytryptamine. This was done exactly as described in Materials and Methods using 33 μg of protein. The dilution media contained: (A) 90 mM NaCl and 10 mM sodium phosphate (pH 6.8), without (○) or with 20 μM 5-hydroxytryptamine (●); (□) 90 mM KCl + 10 mM potassium phosphate (pH 6.8); (B) 0.1 M sodium phosphate (pH 6.8) (△) or 90 mM LiCl + 10 mM lithium phosphate (pH 6.8) (▽), without (open) or with 20 μM 5-hydroxytryptamine (closed symbols).

The ATP-dependent 5-hydroxytryptamine uptake

The ATP-dependent reserpine-sensitive biogenic amine transport systems from a variety of systems such as chromaffin granules, platelet storage organelles and synaptic vesicles have been found to be proton-coupled utilizing the pH gradient created by proton pumping ATPase. Also the ATP-dependent system in the vesicles from RBL seems to belong to this class. Both the proton ionophore CCCP and nigericin, which exchanges protons and other monovalent cations, strongly inhibit ATP-dependent amine transport, while the potassium ionophore valinomycin does not (Fig. 7). It is very likely that the ATP-dependent amine transport in vesicles from RBL is very similar to that from other storage organelles, namely linked to protons. Further support comes from the following experiments. Sodium-independent transport of 5-hydroxytryptamine can be driven by an artificially imposed pH gradient (Fig. 8A). When vesicles, preloaded at pH 6.5, are diluted into a medium of pH 8.5, 5-hydroxytryptamine accumulation occurs which is sensitive to reserpine and to nigericin, which is expected to collapse the pH gradient under these conditions (Fig. 8A). As for

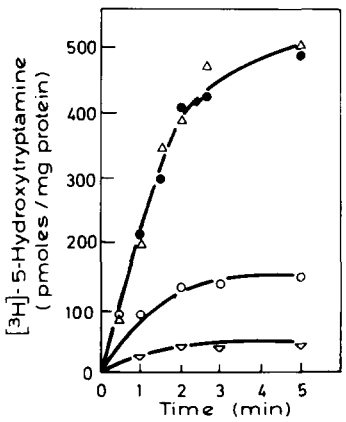

Fig. 7. The effect of ionophores on ATP-dependent 5-hydroxytryptamine transport. In this experiment 2 μCi of the ligand was present and 143 μg of membrane protein was used. Additions: (●) none; (△) valinomycin, 2.5 μM; (○) nigericin, 5 μM; (▽) CCCP, 5 μM.

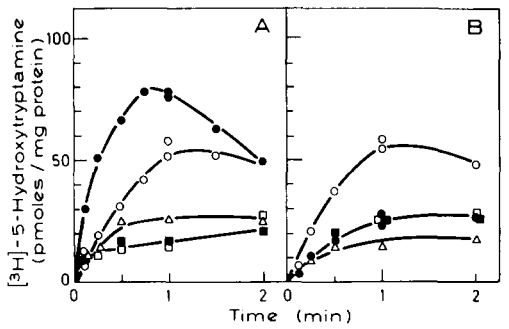

Fig. 8. The effect of valinomycin on ΔpH-driven 5-hydroxytryptamine transport. (A) With inward directed potassium gradient. The internal medium was 250 mM sucrose plus 10 mM sodium Hepes (pH 6.5), while the external solution contained in addition to the labelled solute also 100 mM sucrose and 95 mM potassium Hepes (pH 8.5). Protein, 35 μg (in 5 μl of volume). (B) With outward directed potassium gradient. In this case the same concentrations were used 'in' and 'out' except that the external medium contained sodium and the internal one contained the potassium. In this experiment the amount of protein used was 36.5 μg. The symbols are the same for A and B: (○) control; (●) +valinomycin, 2.5 μM; (□)+ reserpine, 1 μM; (■)+reserpine, 1 μM+valinomycin, 2.5 μM; (△)+nigericin, 5 μM.

amine transport in the chromaffin granules, the process in RBL vesicles is electrogenic as well. With vesicles containing internal sodium and with a potassium containing external medium, addition of valinomycin results in a marked stimulation of reserpine-sensitive 5-hydroxytryptamine transport (Fig. 8A). This condition is expected to create a diffusion potential (interior positive) of the same polarity which an inward directed proton pumping ATPase would yield. Furthermore, reversal of the polarity of the potential induced by valinomycin (potassium inside, sodium outside) does in fact strongly inhibit ΔpH driven 5-hydroxytryptamine accumulation (Fig. 8B). Thus more than one proton antiports with each charged amine.

Recently it was demonstrated that amine efflux from chromaffin granules is regulated by the internal pH [17]. At an acidic interior pH very low efflux occurs, under conditions where the amine should exit. Efflux could be provided by increasing the internal pH or via exchange. A qualitatively similar situation appears to exist in the case of the amine-containing storage organelles from RBL cells, with considerable efflux still occurring without these manipulaions (data not shown). This is also indicated by the experiment depicted in Fig. 1A, where efflux occurs at longer time, as opposed to chromaffin granules [17].

Discussion

We have shown that in RBL cells two types of transport systems for 5-hydroxytryptamine can be identified; a sodium-coupled system located in the plasma membrane and a proton coupled one apparently located in the amine storage organelles. Thus far all amine-secreting cells have both these transport systems in common. As these transport systems work in series, they represent a very efficient machinery to recover any secreted amines and to prevent them from leaking out spontaneously. Since the mechanism of calcium-dependent exocytotic release is different, this results in negligible background release, and thus exocytotic release for signalling can occur at a very high signal to noise ratio.

As noted, both systems are very similar when the various secretory cells are compared. All plasma membrane systems are sodium-dependent and imipramine-sensitive (Refs. 1, 2, 6 and this publication). This system has been investigated in membrane vesicles from platelets (see Ref. 20) and from RBL (this paper). The similarity between the two systems is striking. In addition to imipramine-sensitivity and energization by the sodium gradient, both systems require internal potassium and external chloride for influx. On the other hand, doubt exist on the question of electrogenicity. The platelet system was reported to be electroneutral [19,21]. It was found that addition of valinomycin resulted in a very strong hyperpolarization (8-fold), with little or no stimulation of 5-hydroxytryptamine transport. The RBL system, however, is consistently stimulated by valinomycin. Hence, (1) the systems are different in this regard or (2) the valinomycin experiments are inconclusive. Indeed, the latter very well may be the case. The potassium gradient (in > out) has been suggested to be a driving force for influx of 5-hydroxytryptamine in platelets [18,19]. Addition of valinomycin will have two opposing effects on 5-hydroxytryptamine transport: (1) Stimulation, by enhancing the membrane potential (interior negative); (2) Inhibition, by allowing, in the presence of permeant ions,

faster dissipation of the potassium gradient. A very similar situation exists for the high-affinity glutamate uptake. That system for rat brain uses the potassium ion gradient (in > out) as one of the driving forces and is electrogenic [22,23]. In that case it is not easy to demonstrate the valinomycin effect [22]. A very similar glutamate uptake system exists in the kidney and there is disagreement between two groups on the question of electrogenicity [24,25].

The storage organelle system from RBL cells is apparently coupled to the proton gradient, just as that from chromaffin granules, platelet storage organelles and synaptic vesicles (see Refs. 7, 26). One difference is that the control of amine exit by internal pH is extremely tight in the chromaffin granules [17], and less tight in the other systems [8].

A photo-affinity label for the chromaffin granule system has been developed [27]. Preliminary experiments indicate that this compound also specifically labels the storage organelles from the other sources including the membranes of RBL cells (Gabizon, R., Rudnick, G., Kanner, B.I. and Schuldiner, S., unpublished data). This indicates their similarity.

Acknowledgements

We wish to thank Dr. Henry Metzger for his interest and advice. We are also grateful to Dr. Eli Zlotkin and Mrs. Lena Fishman for performing electron microscopy for us. This study was supported by Grant N.S.16708-01 from the National Institute of Neurology and Commmunicative Disorders and Stroke.

References

1 Iversen, L.L. (1971) Br. J. Pharmacol. 41, 571–591
2 Iversen, L.L. (1973) Br. Med. Bull. 29, 130–135
3 Sneddon, J.M. (1973) Prog. Neurobiol. 1, 151–198
4 Paasonen, M.K. (1968) Ann. Med. Exp. Biol. Fenn. 46, 416–422
5 Tranzer, J.P., Da Prada, M. and Pletcher, A. (1972) Biochem. Biophys. Res. Commun. 46, 1769–1775
6 Rudnick, G. (1977) J. Biol. Chem. 252, 2170–2174
7 Njus, D., Knoth, j. and Zallakian, M. (1981) Curr. Top. Bioenerg. 11, 107–147
8 Maron, R., Kanner, B.I. and Schuldiner, S. (1979) FEBS Lettt. 98, 237–240
9 Toll, L. and Howard, B.D. (1978) Biochemistry 17, 2517–2523
10 Fishkes, H. and Rudnick, G. (1982) J. Biol. Chem. 257, 5671–5677
11 Uvnas, B. (1974) Fed. Proc. 33, 2172–2176
12 Johnson, R.G., Carty, S.E., Fingerhood, B.J. and Scarpa, A. (1980) FEBS Lett. 120, 75–79
13 Fewtrell, C. and Metzger, H. (1981) in Biochemistry of the Acute Allergic Reactions, pp. 295–314, Alan R. Liss, New York
14 Kanner, B.I. and Metzger, H. (1983) Proc. Natl. Acad. Sci. USA 80, 5744–5748
15 Kanner, B.I. (1978) Biochemistry 17, 1207–1211
16 Schuldiner, S., Fishkes, H. and Kanner, B.I. (1978) Proc. Natl. Acad. Sci. USA 75, 3713–3716
17 Maron, R., Stern, Y., Kanner, B.I. and Schuldiner, S. (1983) J. Biol. Chem. 258, 11476–11482
18 Kanner, B.I., Fishkes, H., Maron, R., Sharon, I. and Schuldiner, S. (1979) FEBS Lett. 106, 175–178
19 Nelson, P.J. and Rudnick, G. (1979) J. Biol. Chem. 254, 10084–10089
20 Keyes, S.R. and Rudnick, G. (1982) J. Biol. Chem. 257, 1172–1176
21 Rudnick, G. and Nelson, P.J. (1978) Biochemistry 17, 5300–5303
22 Kanner, B.I. and Sharon, I. (1978) Biochemistry 17, 3939–3953
23 Kanner, B.I. and Sharon, I. (1978) FEBS Lett. 94, 245–248
24 Schneider, E.G. and Sacktor, B. (1980) J. Biol. Chem. 255, 7645–7649
25 Burckhardt, G., Kinne, R., Stange, G. and Murer, H. (1980) Biochim. Biophys. Acta 599, 181–201
26 Kanner, B.I. (1983) Biochim. Biophys. Acta 726, 293–316
27 Gabizon, R., Yetinson, T. and Schuldiner, S. (1982) J. Biol. Chem. 257, 15145–15150