THE ROLE OF A TRANSMEMBRANE pH GRADIENT IN 5-HYDROXY TRYPTAMINE UPTAKE BY SYNAPTIC VESICLES FROM RAT BRAIN

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1. Introduction

In various biological systems, the bulk of biogenic amines is stored in specialised cell organelles. Among the best known examples we find chromaffin vesicles from the adrenal medulla [1,2], 5-hydroxy tryptamine (5-HT) containing granules in blood platelets [3], histamine granules in mast cells [4] and neuronal synaptic vesicles [5]. These vesicles play a central role in storage and release of hormones and neurotransmitters [6]. In addition they are able to actively accumulate biogenic amines [7]. Synaptic vesicles have therefore been implicated in termination of synaptic transmission. The sequence proposed [6] is removal of the neurotransmitters from the synaptic cleft by a high-affinity uptake system, located in the plasma membrane, and their subsequent accumulation in the synaptic vesicles. These two uptake systems differ in their drug sensitivity and ion dependence. While the transport through the plasma membrane is driven by a Na^+ gradient [7], the driving force for accumulation in the synaptic vesicles remains to be clarified.

In chromaffin vesicles from adrenal medulla [8–10] and 5-HT-containing granules from platelets (Rudnick and S.S., in preparation) it has been shown that the transmembrane pH gradient has a role in biogenic amine uptake. In chromaffin granules it has been proposed that a membrane-bound proton-translocating Mg^{2+}-ATPase generates the pH gradient [11,12].

In the light of these observations, we have investigated the role of a pH gradient on 5-HT uptake by synaptic vesicles.

2. Materials and methods

A crude synaptosomal fraction was prepared from rat brain essentially as in [13,14]. The mitochondrial pellet before the Ficoll gradient was washed once more, collected by centrifugation and resuspended in 90 ml final vol. of a solution containing 5 mM Tris–HCl buffer (pH 7.4) and 1 mM potassium EDTA and stirred for 45 min at 0–4°C. After centrifugation of the lysate for 20 min at 20 000 × *g* the pellet was discarded. The crude synaptic vesicle preparation was obtained by centrifugating the supernatant for 60 min at 100 000 × *g*. The pellet obtained was resuspended in a 0.3 M sucrose solution containing 10 mM K-Hepes (pH 7.4) at 5–7 mg protein/ml and stored in liquid air. This preparation was stable for at least 4 weeks.

ATP-dependent transport was assayed at 30°C in 0.2 ml medium containing (final conc.) 0.3 M sucrose, 10 mM potassium Hepes (pH 8.5), 5 mM KCl, 2.5 mM $MgSO_4$, 5 mM ATP and 0.09 μM 5-[1, 2-^{3}H(*N*)]hydroxy tryptamine creatinine sulfate (27.8 Ci/mmol). The reaction was initiated by addition of a small aliquot (10 μl) of membranes vesicles containing 50–70 μg protein. At the indicated times, uptake was terminated and assayed as in [10].

Thin-layer chromatography of the accumulated radioactivity was performed with the material collected from 5-HT uptake at 5 min. The filters were extracted overnight in 2 ml 0.02 M acetic acid at 4°C in the dark. The solvent system used was 15% (v/v) acetic acid, 60% (v/v) in butanol, 25% (v/v) H_2O. Protein was determined as in [15].

5-[1, 2-^{3}H (*N*)]hydroxytryptamine creatinine sulfate (27.8 Ci/mmol) was purchased from NEN. Nigericin was a generous gift of Dr R. J. Hosley of the Eli Lilly Co. All other chemicals were reagent grade obtained from commercial sources.

3. Results and discussion

3.1. *5-HT uptake is ATP-dependent and reserpine-sensitive*

Upon addition of ATP to a suspension of synaptic vesicles, a rapid uptake of 5-HT is observed (fig.1A). This uptake is linear for ~1 min and slowly levels off. The maximum amount of 5-HT accumulated after 5 min is 125 pmol/mg protein^{-1}. Thereafter the 5-HT content of the synaptic vesicles slowly decreases. In the absence of externally added ATP, the level of 5-HT accumulation is only 25% of the uptake in the presence of ATP. Reserpine, a potent inhibitor of ATP-dependent catecholamine transport in chromaffin granules [16] also strongly inhibits the accumulation of 5-HT in the crude synaptic vesicle preparation (fig.1A).

Imipramine, a well-known inhibitor of Na^+-dependent biogenic amine accumulation has no effect on the uptake into synaptic vesicles (table 1). This differential sensitivity as well as the ATP requirement and the

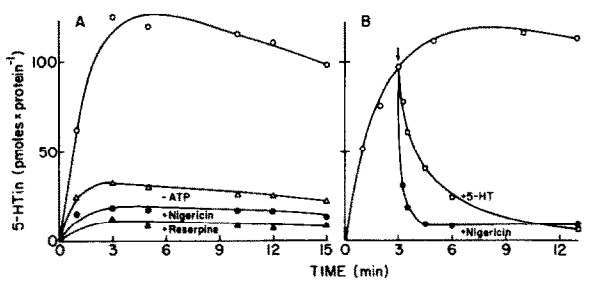

Fig.1A. Effects of ATP, ionophore and inhibitor on 5-[^{3}H]HT transport in synaptic vesicles. Transport assays were performed as in section 2 except that ATP was omitted from one of the reaction media (△——△). No additions (○——○); nigericin, 2.5 μM (●——●); reserpine, 1.25 μM (▲——▲).

Fig.1B. Efflux of 5-[^{3}H]HT from synaptic vesicles. Transport assays were performed as in fig.1A. After 3 min of uptake, the following additions were made: none (○——○); 5-HT, 50 μM (□——□); nigericin, 5 μM (●——●).

Table 1
Differential effects of sodium and imipramine on 5-HT uptake in synaptic vesicles and synaptosomes

Medium composition	Synaptosomal fraction (% control)	Synaptic vesicles
No sodium	9	74
Sodium	100	100
Sodium + imipramine	29	123

The incubation medium contained 127.2 mM NaCl, 5 mM KCl, 2.7 mM $CaCl_2$, 1.3 mM $MgSO_4$, 2.5 mM Tris (pH 7.4), 0.09 μM 5-[^{3}H]HT. In addition 11.1 mM D-glucose was present in the synaptosomal assay medium and 5 mM ATP was added in the case of synaptic vesicles. For uptake in the absence of sodium, NaCl was replaced by an equimolar amount of KCl. Imipramine, when present, was added to 2 μM final conc. Assays of the synaptosomes were performed within 45 min of fractionation [23]. Control uptake values were 14 pmol/mg protein/min for synaptosomes and 5.7 pmol/mg protein/min for synaptic vesicles

Na^+ independence of the latter process (table 1) support the contention that the uptake observed is due to the activity of synaptic vesicles.

When an excess of unlabelled 5-HT is added, after 3 min of uptake, a rapid efflux of the pre-accumulated radioactivity is observed (fig.1B). This radioactivity represents unmodified 5-HT since > 70% of the radioactivity co-migrates with authentic 5-HT on thin-layer chromatography.

Assuming an internal volume of ~3 μl/mg protein [17] and maximal uptake of 140 pmol 5-HT/mg protein, the vesicles concentrate 5-HT at least 500-fold. This value is most probably an underestimate since it is likely that only a fraction of the vesicle population is active in 5-HT uptake.

3.2. *Effect of ionophores on 5-HT uptake*

In chromaffin granules it appears that it is the ΔpH component of the protonmotive force generated by the membrane ATPase, which is the immediate driving force for the accumulation of biogenic amines [8–10,18]. In order to determine whether the same kind of mechanism operates in synaptic vesicles from rat brain, we have examined the effect of ionophores on the transport process.

Nigericin, known to collapse ΔpH by exchanging

K^+/H^+, strongly inhibits 5-HT uptake (fig.1A). Moreover, when added after 3 min of uptake it induces a rapid efflux of pre-accumulated 5-HT (fig.1B). On the other hand, valinomycin, collapsing $\Delta\Psi$ under our conditions, does not significantly inhibit 5-HT uptake (data not shown). Qualitatively similar results are also obtained with either radioactive epinephrine or norepinephrine (data not shown). Therefore, it is suggested that also in synaptic vesicles, biogenic amine transport is driven by a pH gradient and that $\Delta\Psi$ does not significantly contribute to the uptake. This conclusion is supported by a study on norepinephrine uptake in synaptic vesicles [19].

3.3. *Kinetic constants and pH profile of 5-HT uptake*

The pH profile of the ATP-dependent uptake in synaptic vesicles (fig.2) is remarkably similar to the analogous process in chromaffin granules [20]. Thus, in both systems the pH optimum as ~pH 8.3. As with chromaffin granules, this preparation is capable of accumulating amines such as norepinephrine ([19], R.M. unpublished observations), dopamine [21], epinephrine (R.M. unpublished observations) and 5-HT. In the former preparation it was suggested that the uptake of the different amines is mediated by a single carrier protein. Since the synaptic vesicles are not homogeneous and their amine content is unknown, a kinetic approach does not lead to unequivocal conclusions. 5-HT uptake is a saturable process. The app. K_m is 1.0 μM and the V_{max} 212.0 pmol/mg protein/min. Norepinephrine is a competitive inhibitor of this uptake with app. K_i 1.2 μM. This K_i is similar to the app. K_m reported for norepinephrine uptake [19].

The results presented here and in [19] support the contention that biogenic amine uptake in synaptic vesicles is driven by a transmembrane pH gradient. Direct support for the above notion may be achieved by imposition of artificial pH gradients. However, such experiments are not feasible when only a small percentage of the population contains a reserpine-sensitive carrier, since amines may equilibrate with a pH gradient even in the absence of a carrier [22]. Thus the direct demonstration of the role of a pH gradient in 5-HT uptake will have to await the further purification of the vesicles.

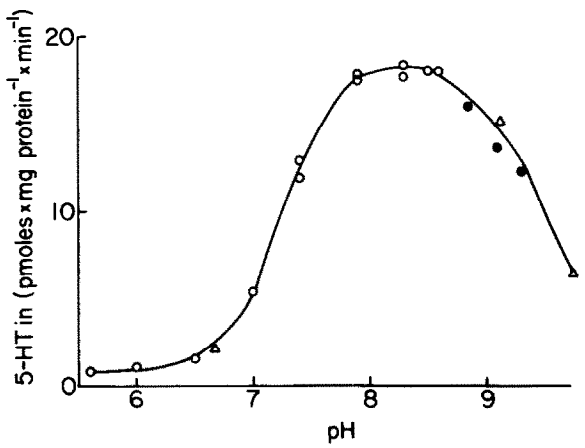

Fig.2. pH profile of 5-[^{3}H]HT transport in synaptic vesicles. Assays were performed as in fig.1A for 3 min except that the medium pH was as indicated and the buffers used (10 mM) were, K-Hepes (○——○); K-Tris hydroxymethyl methylaminopropane sulfonate (●——●); K-cyclohexylaminopropane sulfonate (△——△).

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