RESERPINE AS A COMPETITIVE AND REVERSIBLE INHIBITOR OF THE CATECHOLAMINE TRANSPORTER OF BOVINE CHROMAFFIN GRANULES

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1. Introduction

The catecholamine-rich organelles of adrenal medulla, the chromaffin granules, are involved in transport, storage and secretion of these biogenic amines [1–4]. The isolated granules catalyse the uptake of large amounts of adrenaline and other biogenic amines by an ATP-dependent [5], uncoupler-sensitive [6] process. The granules have been found to contain a membrane-bound ATPase [7–10]. The latter enzyme has been shown to translocate protons and to create an electrochemical proton gradient [11–16]. Moreover, indirect evidence has been obtained for the involvement of the pH gradient (ΔpH) component [14] as well as the membrane potential ($\Delta\psi$) component of this gradient [17] in amine transport. The ability to drive amine transport by artificially imposed pH gradients (acidic inside) provides direct evidence for the participation of at least ΔpH as an immediate driving force for the process [18,19].

The ability of the granules [5] and isolated granule membrane vesicles [20] to accumulate various biogenic amines, as well as the mutual inhibition on transport of the amines [5] suggests the presence of a single carrier with a rather broad specificity. This may also serve to explain the inhibitory action of reserpine which also has been reported to act as a competitive inhibitor of the transport process [21]. In apparent contrast to this observation is the reported irreversibility of this drug (cf. [22]).

Using isolated membranes lacking endogenous amines, we provide here additional evidence for a single translocator for biogenic amines in the chromaffin granule membrane. Moreover, we show that reserpine indeed acts as a competitive inhibitor and that its suggested irreversibility is only apparent, due to the hydrophobicity of the drug.

2. Materials and methods

2.1. Chemicals

D,L-[7-^{3}H]adrenaline hydrochloride (10.3 Ci/mmol), D,L-[7-^{3}H]noradrenaline hydrochloride (11 Ci/mmol) and 5-hydroxy [G-^{3}H]tryptamine (0.5 Ci/mmol) were purchased from Amersham, aselectin was from Associated Concentrates, Woodside NY. Unlabelled biogenic amines and all other chemicals were from commercial sources and of the highest purity available.

2.2. Chromaffin granule membrane vesicles

These were isolated as in [19].

2.3. Transport assays

ATP-dependent transport and ΔpH-dependent transport were assayed as in [19]. When biogenic amines other than adrenaline were assayed, their final concentrations were (11 Ci/mmol) 0.22 μM D,L-[7-^{3}H]noradrenaline and 5 μM (0.5 Ci/mmol) 5-hydroxy [G-^{3}H]tryptamine (serotonin).

2.4. Protein determinations

These were as in [23].

3. Results and discussion

The rate of ATP-dependent adrenaline transport in chromaffin granule membrane vesicles is dependent on the external solute concentration and is a saturable function. When the data are plotted according to Lineweaver-Burk, the app. V_{max} for noradrenaline and serotonin uptake are 4.9 and 1.8 μmol/min.mg protein, respectively and the respective app. K_m values are 12.5 μM and 4.3 μM (table 1). The app. K_m values are in good agreement with the affinity constants determined [20]. Transport of adrenaline, noradrenaline and serotonin is competitively inhibited by each of the other amines as well as by dopamine and reserpine (table 1). In the case of adrenaline, noradrenaline and serotonin the app. K_m of each transport substrate correlates very well with its apparent inhibition constant on the transport of the other two amines. These data support the notion that a single carrier is responsible for the transport of the different amines in this preparation.

The ATP-dependent amine transport is very likely the result of two sequential processes:

(i)   Generation of a proton electrochemical gradient by the membrane-bound ATPase;

(ii)   Utilisation of (at least) the ΔpH component to drive the carrier-mediated accumulation. Therefore, uptake driven by an artificially imposed

Table 2
Effect of reserpine and catecholamines on pH-driven adrenaline transport

Additions	Adrenaline uptake (pmol/mg protein)
None	14.2
L-Noradrenaline, 50 μM	4.5
Dopamine, 50 μM	3.2
Reserpine, 1 μM	0.8

ΔpH-driven transport was measured as in section 2. The reaction was initiated by addition of 10 μl membrane vesicle suspension (45 μg protein) and terminated after 1 min. Inhibitors were added 30 s prior to the membranes

ΔpH should also be inhibited by other biogenic amines.

As shown in table 2, adrenaline accumulation induced by ΔpH is, like ATP-dependent transport, inhibited by reserpine, dopamine and noradrenaline (table 2). Therefore, it is concluded that the ΔpH-driven process is carrier mediated as well.

Reserpine has been reported to inhibit catecholamine uptake into subcellular storage vesicles in various preparations such as those from brain tissue and the adrenal medulla [5,22]. Reserpine was shown to behave [21] as a competitive inhibitor of adrenaline transport in the adrenal medullary vesicles. However, other

Table 1
Apparent K_m and K_i values of biogenic amine transport

Inhibitor	Apparent inhibition constants (μM) of the transport of:		
	Adrenaline	Noradrenaline	Serotonin
L-Adrenaline	(18.5)	19.4	20.1
L-Noradrenaline	14.1	(12.5)	13.3
Serotonin	2.8	2.9	(4.3)
Dopamine	8.7	6.2	6.8
Reserpine	0.016	n.d.	0.019

ATP-dependent transport was measured as in section 2. The reaction was initiated by addition of 10 μl membrane vesicle suspension (30–50 μg protein) and terminated after 3 min. The data were plotted according to Lineweaver-Burk and the appropriate constants were calculated. The data in parenthesis are the app K_m values. Since in the case of adrenaline and noradrenaline D,L mixtures were used, the app. K_m values should be corrected according to [27]. This is not likely to change the good agreement between the K_m and K_i values. For instance, the K_m for noradrenaline corrected [27] using the affinity constants for the D and L isomer yields a value of 15.6 μM
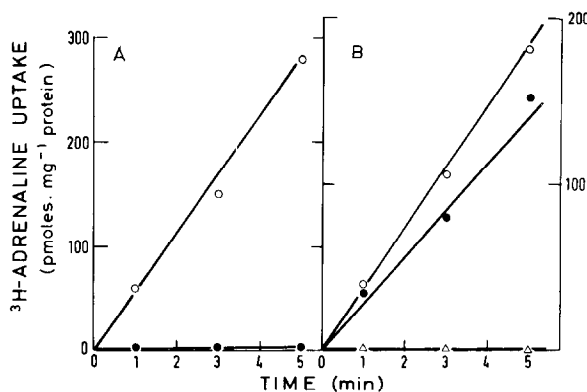
Fig.1. Reversibility of the reserpine inhibition on adrenaline transport. Chromaffin granule membrane vesicles (0.5 mg protein/ml) were incubated at 37°C in a medium containing 0.3 M sucrose, 10 mM K-Hepes (pH 8.5), 2.5 mM $MgSO_4$ and 5 mM KCl in the presence or absence of 0.6 μM reserpine. After 5 min incubation, aliquots were taken to assay ATP-dependent adrenaline accumulation, and remaining membranes were diluted 10-fold in a medium of the same composition without reserpine but containing liposomes (1.3 μmol lipid/ml) and further incubated at 37°C. After 5 min, the suspension was centrifuged at 40 000 × g for 20 min. The pellet was resuspended in the above liposome--containing medium and the above-described incubation and centrifugation procedure was repeated twice. Finally, the pellet was resuspended and assayed as described. In parallel experiments the phospholipids were omitted from the incubation and washing medium. Liposomes were prepared from asolectin using a bath-type sonifier. (A) Unwashed membrane vesicles: Control (o- - -o), + 0.6 μM reserpine (o- - -o). (B) Membrane vesicles treated with reserpine (o- - -o, ᐃ- - -ᐃ) and washed in the presence (o- - -o) or in the absence (ᐃ- - -ᐃ) of liposomes. Washed vesicles not treated with reserpine (●- - -●).

observations suggested that the inhibition is irreversible (reviewed in [22]). Our kinetic data on the isolated chromaffin granule membranes support the contention that reserpine competitively inhibits the transport system. The app. K_i (16 nM) is in good agreement with the K_i value obtained with the intact granules [21]. The action of reserpine in the isolated membranes also seemed to be irreversible (fig.1). Thus, pretreatment of the membranes with 1 μM reserpine completely inhibits adrenaline transport, even after three consecutive washings. A possible explanation for the apparent irreversibility of the action of this compound might be its highly hydrophobic nature. Indeed, when phospholipid vesicles are included in

the washing medium, an almost complete reversal of the inhibition by reserpine is observed (fig.1B). It should be pointed out that the transport activity after reversal is still reserpine sensitive (data not shown). According to our hypothesis, the phospholipid vesicles provide an hydrophobic milieu which is able to remove the inhibitor from the chromaffin granule membranes.

The mechanism of accumulation described for the chromaffin granule may well apply to other subcellular organelles that store biogenic amines. For instance, in certain neuronal preparations [24] and platelets [25], reserpine induces the release of catecholamines and 5-hydroxytryptamine, respectively, but does not inhibit uptake through the plasma membrane [26]. These effects of reserpine may reflect the presence of two types of transport systems for biogenic amines:

(i) An Na^+-dependent carrier for translocation across the plasma membrane that is insensitive to reserpine;

(ii) An H^+-coupled system for accumulation across the storage organelle membrane that is inhibited by reserpine.

Acknowledgements

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