5-Hydroxytryptamine transport in cells and secretory granules from a transplantable rat insulinoma

John C. HUTTON,* Mina PESHAVARIA and Nigel E. TOOKE

Department of Clinical Biochemistry, University of Cambridge, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QR, U.K.

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Mechanisms of transport of 5-hydroxytryptamine in the pancreatic B-cell were investigated by using cell suspensions and secretory granules prepared from a transplantable rat insulinoma. (1) Cells incubated with 5-hydroxy[G-³H]tryptamine at concentrations ranging from 0.1 μ M to 5 mM accumulated the radioisotope principally by a simple diffusion process. The incorporated radioactivity was recovered principally as the parent molecule and was recovered predominantly in soluble protein and secretory-granule fractions prepared from the tissue. (2) Isolated granules incubated in buffered iso-osmotic medium without ATP accumulated the amine to concentrations up to 38-fold that of the medium. This process was insensitive to reserpine and occurred over a wide range of 5-hydroxytryptamine concentrations (0.075 μ M–25 mM). Above 5 mM, 5-hydroxytryptamine accumulation decreased in parallel with the breakdown of the Δ pH across the granule membrane. Uptake was favoured by alkaline media and was reduced by the addition of (NH₄)₂SO₄. In both cases a close correlation was observed between uptake and the transmembrane Δ pH, a finding that suggested that 5-hydroxytryptamine permeated the membrane as the free base and equilibrated across the membrane with the Δ pH. Binding of 5-hydroxytryptamine to granule constituents also played a part in this process. ATP caused a further doubling of granule 5-hydroxytryptamine uptake by a process that was sensitive to reserpine (0.5 μ M). Inhibitor studies suggested that amine transport in this instance was linked to the activity of the granule membrane proton-translocating ATPase. (3) It was concluded that the uptake of amines driven by proton gradients across the insulin-granule membrane could account for the accumulation *in vivo* of amines in the B-cell.

Pancreatic B-cells, in common with many endocrine cells that secrete polypeptide hormones, accumulate the biogenic amines 5-hydroxytryptamine and dopamine within their secretory granules (Falck & Hellman, 1963; see Owman *et al.*, 1973). Marked species variation is encountered in the intragranular level of these compounds as assessed by histochemical and ultrastructural assays (see Owman *et al.*, 1973), a phenomenon that may in part be related to differences in the presentation of amines to the B-cell from the circulation or from cells associated with the islet. The administration *in vivo* of amine precursors in conjunction with a monoamine oxidase inhibitor thus increases tissue levels

* To whom correspondence and reprint requests should be sent.

Abbreviations used: Δ pH, pH gradient; $\Delta\psi$, membrane potential, $\Delta\mu_{\text{H}^+}$, protonmotive force; Hepes, 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid.

by almost two orders of magnitude (Bird *et al.*, 1980) and can reveal intragranular accumulation of amines in species in which endogenous amines cannot normally be detected histochemically (Cegrell, 1970).

Investigation of the net uptake of 5-hydroxytryptamine radioactivity by intact islets and by dispersed islet cells from *ob/ob* mice has suggested the functioning in the B-cell of a high-affinity Na⁺-dependent 5-hydroxytryptamine transport system of low capacity together with a low-affinity non-saturable transport process (Lindstrom *et al.*, 1980; Lindstrom, 1981). Considerable variation is evident in the uptake rates in different species (Mahoney & Feldman, 1977). The radioactivity incorporated by *ob/ob* mouse islets is recovered after disruption of the tissue principally in a soluble protein fraction and a particulate fraction containing insulin granules, mitochondria and lysosomes

(Hellman *et al.*, 1972). Efflux of radioactivity from perfused islets pre-labelled with radioactive 5-hydroxytryptamine is enhanced by glucose and 3-isobutyl-1-methylxanthine, a finding that suggests that intragranular 5-hydroxytryptamine may be co-released with insulin (Gylfe, 1978).

Interpretation of studies of 5-hydroxytryptamine transport in whole islets is complicated by the cellular heterogeneity of the tissue, metabolism of the amine and by the participation of at least two membrane systems in the process, namely the plasma membrane of the cell and the granule envelope. The present paper describes the uptake of 5-hydroxytryptamine by isolated insulin secretory granules and B-cells prepared from a transplantable rat insulinoma. The principal objective here was to document the relationship between amine transport and the chemiosmotic properties of the granule.

Experimental

5-Hydroxy[G-³H]tryptamine creatinine sulphate (sp. radioactivity 10 Ci/mmol), 5-hydroxy[*side chain*-¹⁴C]tryptamine creatinine sulphate (sp. radioactivity 60 Ci/mol), [¹⁴C]methylamine hydrochloride (sp. radioactivity 60 Ci/mol), potassium thio[¹⁴C]cyanate (sp. radioactivity 60 Ci/mol), inulin[¹⁴C]carboxylic acid (sp. radioactivity 6 Ci/mol), [U-¹⁴C]sucrose (sp. radioactivity 10 Ci/mol) and ³H₂O (5 Ci/ml) were obtained from Amersham Int., Amersham, Bucks., U.K. Tri-*n*-butyltin chloride (tributyltin) was purchased from Ralph N. Emanuel, Alpertown, Middx., U.K., and other biochemical reagents were from Sigma (London) U.K., Kingston upon Thames, Surrey, U.K. Nigericin was a gift from Roche Products, Welwyn Garden City, Herts., U.K.

Insulinoma tissue derived from an X-ray-induced tumour (Chick *et al.*, 1977) was propagated in NEDH rats and harvested as described previously (Hutton *et al.*, 1981). Cell suspensions were prepared by digesting tumour pieces (about 2 g total wet wt.) for 30 min at 37°C in 3 ml of Krebs-Henseleit bicarbonate buffer (Dawson *et al.*, 1978) containing 5.6 mM-glucose, 5 mg of bovine plasma albumin/ml (Armour Pharmaceuticals, Eastbourne, Sussex, U.K.), 2.5 mg of collagenase/ml (Boehringer Corp., Lewes, Sussex, U.K.) and 0.1 mg of deoxyribonuclease I/ml. The cells were then isolated by centrifugation for 3 min at 300g, washed twice in medium without collagenase and filtered through 500 µm and then 50 µm sieves (Pharmacia, Uppsala, Sweden). They were then layered on to 5 ml of a solution (pH 7.4) containing 60% (v/v) Percoll (Pharmacia), 150 mM-NaCl and 10 mM-Hepes and centrifuged in an 8 × 2.5 cm tube at room temperature for 30 min at 900g (*r*_{av} 12 cm). Cells were recovered from the interface and washed twice in

fresh medium before use. The viability of the final preparation assessed from Trypan Blue exclusion was between 80 and 95%.

Insulin secretory granules were prepared from homogenates of tumour tissue by density gradient centrifugation using Percoll-containing medium (Hutton & Peshavaria, 1982; Hutton *et al.*, 1982). The final preparation was suspended at 10 mg of protein/ml in a solution containing 0.25 M-sucrose and 10 mM-4-morpholine-ethanesulphonic acid that had been adjusted to pH 6.5 with Tris base. Granules were maintained at 4°C and used within 60 min of their isolation. Fluorimetric assays (Bogdanski *et al.*, 1956) showed that the endogenous 5-hydroxytryptamine content of the prepared material was less than 20 pmol/mg of protein.

Cellular uptake studies

Cell suspensions (5 mg of protein/ml) were pre-incubated for 20 min at 37°C under an O₂/CO₂ atmosphere (19:1, v/v) in 100 µl of a buffer containing 115 mM-NaCl, 5 mM-KCl, 1 mM-MgCl₂, 2.5 mM-CaCl₂, 24 mM-NaHCO₃ and 1 mg of bovine plasma albumin/ml. 5-Hydroxy[G-³H]tryptamine (0.5–20 µCi/ml) combined with unlabelled 5-hydroxytryptamine was then added and the incubation was continued for a further 30 min. Incubations were terminated by adding 1 ml of ice-cold medium and filtering the cell suspension on 5 µm pore-size Millipore type SMWP 025 filters (Millipore Corp., Bedford, MA, U.S.A.). The filters were washed twice with 5 ml of ice-cold medium then transferred to 5 ml of a toluene/Triton X-100-based scintillation counting mixture (Fisofluor 2; Fisons, Loughborough, Leics., U.K.) for the determination of radioactivity by liquid-scintillation spectrometry. Blank corrections were made either by incubating cells with the radioisotopes for <10 s or by omitting cells from the incubation. In both cases this was equivalent to 2–5% of the cellular uptake after 30 min. Compounds of low aqueous solubility were added in 100-fold concentrated solutions in dimethyl sulphoxide; the solvent did not affect any of the parameters currently investigated.

Subcellular localization of radioactivity

Tumour pieces (2 mm diameter; 0.5 g wet wt.) were incubated for 20 min at 37°C in the above medium containing 50 µM-5-hydroxy[G-³H]tryptamine (20 µCi/ml) and 5 µCi of [U-¹⁴C]sucrose/ml as an extracellular marker. The tissue was then rinsed twice in ice-cold medium without radioisotopes, homogenized in a solution (pH 6.5) containing 0.25 M-sucrose, 10 mM-4-morpholine-ethanesulphonic acid/Tris and 1 mM-EGTA. The homogenate was subjected to centrifugation to remove nuclei and unbroken cells, then fractionated on a Percoll density gradient, a procedure identical with

that used in the preparation of granules (Hutton *et al.*, 1982). Samples (0.5 ml) of fractions from the gradient were mixed with 5 ml of scintillation fluid and their radioactivities were determined as described above. The insulin and protein contents of samples were determined as described in the original method.

Determination of 5-hydroxytryptamine uptake and chemiosmotic parameters in isolated granules

5-Hydroxytryptamine uptake by isolated granules was determined by a method similar to that used to ascertain the ΔpH and $\Delta\psi$ across the granule membrane (Hutton, 1982). Suspended granules (0.5 mg of protein/ml) were incubated normally for 20 min at 37°C in medium containing 0.25 M-sucrose, 25 mM-Hepes, 1 mM-MgSO₄, 1 mM-EGTA and various other additions as indicated. The microcentrifuge tubes (1.5 ml-capacity Eppendorf tubes; W. Sarstedt, Beaumont Leys, Leics., U.K.) in which the incubations were performed were pretreated with a solution of 2% (w/v) bovine plasma albumin and rinsed with water to reduce non-specific binding of the radioisotopes. 5-Hydroxy[¹⁴C]tryptamine (0.4 $\mu\text{Ci/ml}$), [¹⁴C]-methylamine (1 $\mu\text{Ci/ml}$), thio[¹⁴C]cyanate (1 $\mu\text{Ci/ml}$) or inulin[¹⁴C]carboxylic acid (2.5 $\mu\text{Ci/ml}$) were added to the incubation medium in conjunction with ³H₂O (5 $\mu\text{Ci/ml}$). 5-Hydroxy[G-³H]tryptamine (0.75 $\mu\text{Ci/ml}$) was combined with inulin[¹⁴C]carboxylic acid (2.5 $\mu\text{Ci/ml}$) when used.

Incubations were terminated by centrifugation for 3 min at room temperature at 9000 g (Beckman Microfuge B; Beckman Instruments, Palo Alto, CA, U.S.A.). A 1:1 (v/v) mixture (300 μl) of dibutyl phthalate and silicone fluid MS 550 (BDH Chemicals, Poole, Dorset, U.K.) was added to each tube and a further 10 s centrifugation was performed. The tube tip was then removed with a scalpel, transferred to 0.5 ml of water and its radioactivity was determined after addition of 5 ml of scintillation cocktail by dual-channel liquid-scintillation spectrometry as described above. The extragranular water space represented by the recovery of radioactivity from inulin[¹⁴C]carboxylic acid in the pelleted granules was subtracted from the apparent spaces of distribution of the other radioisotopes to calculate their net uptakes. The internal water space of the granule derived in this way was used to calculate the apparent intragranular concentrations of the other radioisotopes. The ratios of the intragranular methylamine and thiocyanate concentrations to their concentrations in the medium were used to calculate the ΔpH and $\Delta\psi$ respectively across the granule membrane. The $\Delta\mu_{\text{H}^+}$ was the sum of these components calculated in mV terms (see Rottenberg, 1979). The binding of radioisotopes to granules was evaluated in incubations in which the

medium osmolarity was reduced by the omission of sucrose. The intragranular water space, methylamine uptake and thiocyanate uptake were negligible under these conditions. 5-Hydroxytryptamine binding, however, was equivalent to 17–19% of the net 5-hydroxytryptamine uptake observed in intact granules incubated in the absence of ATP. Binding appeared to increase as a linear function of 5-hydroxytryptamine concentration in the medium and was not affected by ATP addition. The results of uptake studies performed with 5-hydroxy[G-³H]tryptamine and 5-hydroxy[¹⁴C]tryptamine were indistinguishable. This indicated that neither the uptake of these radioisotopes by intact granules nor the binding phenomenon were artefacts resulting from the presence of minor radioisotopic contaminants.

Granule protein was determined after precipitation with 5% (w/v) trichloroacetic acid by the method of Lowry *et al.* (1951) with bovine plasma albumin as standard. The statistical significance between differences of means ($\pm\text{S.E.M.}$) were estimated by Student's *t* test for unpaired data obtained from three or more different granule preparations. Probability estimates of $P < 0.05$ were taken as evidence of non-random variation in the results.

Results

5-Hydroxytryptamine uptake by cell suspensions

More than 95% of the cells prepared from the tumour tissue by the collagenase digestion procedure were identified as B-cells as evaluated from their immunohistochemical properties (I. G. M. Brons & N. E. Tooke, unpublished work). Their content of insulin was 1.4 ± 0.1 units/mg of protein (four preparations) a value approx. 3-fold that of the tumour as a whole. Rat islet tissue in which about 65% of all cells are B-cells, by comparison, contains approx. 2–3 units/mg.

The isolated tumour cells accumulated 5-hydroxytryptamine in a progressive manner during a 45 min incubation (Fig. 1a). A steady state was not achieved within this time interval and the apparent intracellular concentration attained (20–40 $\mu\text{mol/litre}$ of total cellular water) was lower than the medium concentration (50 μM). The chemical nature of the radioactivity incorporated after a 30 min incubation with 50 μM -5-hydroxy[G-³H]tryptamine was investigated by extracting cells with 0.1 M-HCl followed by t.l.c. on cellulose-coated glass plates using a solvent system of butan-1-ol/water/acetic acid (12:5:3, by vol.). Of the radioactivity incorporated, 77% had a chromatographic mobility identical with that of an authentic 5-hydroxytryptamine standard (R_F 0.45). One other minor constituent (R_F 0.9) comprising 12% of the radioactivity taken up was also apparent.

Fig. 1. Time course and concentration-dependency of 5-hydroxytryptamine uptake by tumour cell suspensions (a) Time course of 5-hydroxy[G- 3 H]tryptamine uptake was followed at a 50μ M concentration of the radioisotope. (b) The concentration-dependency was determined over a 30 min incubation by using 5-hydroxy[G- 3 H]tryptamine combined with unlabelled 5-hydroxytryptamine to achieve the indicated concentrations. Results are displayed on either a double-logarithmic or a linear scale (insert).

Uptake determined over a 30 min incubation period was a linear function of the extracellular 5-hydroxytryptamine concentration over a wide range (0.1μ M– 5 mM; Fig. 1b). This suggested that the major process observed here was one of passive diffusion. At a 0.2μ M extracellular 5-hydroxytryptamine concentration, uptake was reduced to 11% of the control value by reducing the incubation temperature to 0° C. This indicated that superficial binding of the tracer did not account for the net

Fig. 2. Density gradient centrifugation of homogenates of cells pre-labelled with 5-hydroxy[G- 3 H]tryptamine A nuclei- and cell-free homogenate of pre-labelled tumour cells was layered in zone C on to a 27% (v/v) iso-osmotic Percoll solution and centrifuged at 4° C for 1 h and 40 000 g to form the gradient. The radioactivity derived from 5-hydroxy[G- 3 H]tryptamine (b), and the insulin (O) and protein (●) contents (a) of fractions are expressed relative to the maximal value attained on the gradient. 5-Hydroxytryptamine uptake in the experiment shown was 165 pmol/mg of tissue protein.

cellular uptake observed. Veratridine (50μ M), ouabain (0.5 mM) and carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (5μ M), agents that inhibit the Na^+ - and energy-dependent amine transport in other cells, did not significantly affect uptake determined at 0.2μ M-5-hydroxy[G- 3 H]tryptamine. Reserpine, which inhibits vesicular uptake and depletes amine content in a variety of cell types, reduced the insulinoma cell 5-hydroxy[G- 3 H]tryptamine uptake when tested at a concentration of 50μ M. Uptake in the presence of 0.5μ M and 50μ M concentrations of 5-hydroxytryptamine was reduced to $74 \pm 12\%$ ($n = 4$) and $75 \pm 8\%$ ($n = 3$) of the respective control values. No effect of reserpine at a 5μ M concentration, however, was apparent.

Subcellular localization

Subcellular localization studies performed with tumour pieces revealed that $62 \pm 2\%$ ($n = 3$) of the radioactivity incorporated at an extracellular 5-hydroxytryptamine concentration of 50μ M was associated with a soluble protein fraction (zone C; Fig. 2). The remainder was found predominantly in

Fig. 3. Time course and concentration-dependency of 5-hydroxytryptamine uptake by intact granules and the effect of MgATP

Isolated insulin granules were incubated at pH 7 in iso-osmotic medium as described in the Experimental section. Uptake of radioactivity was followed by using either ^{14}C -labelled (Δ and $\blacktriangle$) or ^3H -labelled ($\circ$ and $\bullet$) tracer. Incubations were performed in either the presence (filled symbols) or the absence (open symbols) of 3 mM-MgATP^{2-} . In (a) the time course was followed at $6.6\text{ }\mu\text{M}$ -5-hydroxytryptamine and uptake expressed as the apparent intragranular amine content. In (b), the concentration-dependency was followed over a 20 min incubation period; the results are expressed as the ratio of the apparent intragranular concentration to that of the medium.

fraction was 5.9-fold greater than that of the other particulate fraction (zone B).

5-Hydroxytryptamine uptake by isolated secretory granules

Isolated insulin secretory granules incubated in iso-osmotic medium with $6.6\text{ }\mu\text{M}$ -5-hydroxy[^{14}C]-tryptamine showed a progressive accumulation of radioisotope (Fig. 3). The initial rate was not affected by the addition of ATP to the medium but an effect of this nucleotide was evident on the net accumulation of radioactivity from 10 min onwards. By 50 min the apparent intragranular concentration of 5-hydroxytryptamine was 38-fold that of the medium when ATP was omitted and 60-fold in the presence of ATP.

Net accumulation of 5-hydroxytryptamine by granules occurred over a wide range of external concentrations of the amine both in the presence and absence of exogenous ATP (Fig. 3b). The stimulatory effect of ATP on 5-hydroxytryptamine uptake expressed relative to the control incubation was not markedly affected by variation in the 5-hydroxytryptamine concentration within the range $0.1\text{--}10\text{ }\mu\text{M}$, but thereafter decreased. At higher extragranular 5-hydroxytryptamine concentrations uptake expressed as the ratio of the intragranular concentration to that of the medium decreased progressively both in the presence or absence of ATP. This effect was accompanied by a progressive decrease in the ΔpH and an increase in the $\Delta\psi$ across the membrane (Table 1). In the presence of ATP the $\Delta\mu_{\text{H}^+}$ was not affected, a finding that suggested that H^+ within the granule was being replaced by a chemical species with an equivalent charge, conceivably the cationic form of 5-hydroxytryptamine.

pH dependency

Granular uptake of 5-hydroxytryptamine increased progressively with increasing extragranular pH within the range pH 6.0 to pH 7.4. ATP increased uptake at alkaline pH values but its effect was markedly reduced at pH 6.0 or 6.5. Previous investigations (Hutton, 1982) have shown that the ΔpH across the granule membrane increases from a value of approx. 0.8 at pH 6.0 to 1.3 at pH 7.4, but is not affected by ATP addition under these conditions. The $\Delta\psi$ recorded in the presence of ATP on the other hand falls from a value of 80 mV at pH 6.0 to <10 mV at pH 7.4.

Inhibitor studies

Significant reduction in 5-hydroxytryptamine uptake by isolated granules incubated without ATP was achieved by adding a permeant base in the form of $(\text{NH}_4)_2\text{SO}_4$ or by osmotic lysis (Table 2). The ATP-stimulated increment in uptake was similarly

fractions containing insulin granules (zone A) with a second minor component associated with fractions known to contain most of the mitochondria, endoplasmic reticulum and plasma membranes of the tissue (Hutton & Peshavaria, 1982; Hutton *et al.*, 1982). The specific radioactivity of the peak granule

Table 1. *Effect of reserpine and high concentrations of 5-hydroxytryptamine on the chemiosmotic properties of isolated granules*

The ΔpH , $\Delta\psi$, $\Delta\mu_{\text{H}^+}$ and uptake of ^3H -labelled 5-hydroxytryptamine were determined at pH 7 after a 20 min incubation (see the Experimental section). MgATP was present, where indicated, at 3 mM.

Additions	5-Hydroxytryptamine uptake (nmol/mg)	ΔpH	$\Delta\psi$ (mV)	$\Delta\mu_{\text{H}^+}$ (mV)
None (–ATP)	—	1.09	<0.0	—
1 μM -Reserpine (–ATP)	—	1.00	<0.0	—
5-Hydroxytryptamine (–ATP)				
0.66 mM	29	0.94	<0.0	—
5.0 mM	181	0.66	1.8	41
25.0 mM	379	0.26	21.4	37
None (+ATP)	—	1.14	46.2	115
1 μM -Reserpine (+ATP)	—	1.16	45.7	115
5-Hydroxytryptamine (+ATP)				
0.66 mM	26	1.22	40.7	114
5.0 mM	195	0.86	70.0	122
25.0 mM	483	0.41	86.0	111

Table 2. *Inhibitor studies with intact granules*

5-Hydroxytryptamine uptake by isolated granules was determined at pH 7 over a 20 min incubation in the presence of 6.6 μM -5-hydroxy[^{14}C]tryptamine. The net accumulation of radioactivity is also expressed as a percentage of the control incubation at the same ATP concentration. Abbreviation: FCCP, carbonyl cyanide *p*-fluoromethoxyphenylhydrazone.

Treatment	5-Hydroxytryptamine net uptake			
	Control		Plus ATP	
	(nmol/mg)	(%)	(nmol/mg)	(%)
None	0.34	100	0.61	100
20 mM- K_2SO_4	0.28	82	0.46	75
Plus 0.1 μM -nigericin	0.32	94	0.27	44
Plus 2.5 μM -valinomycin	0.32	94	0.52	85
Plus 0.1 μM -nigericin and 2.5 μM -valinomycin	0.25	74	0.26	43
40 mM-KI	0.30	88	0.52	85
20 mM- $(\text{NH}_4)_2\text{SO}_4$	0.13	38	0.22	36
2.5 μM -Tributyltin	0.32	94	0.33	54
2.5 μM -FCCP	0.32	94	0.33	54
0.5 μM -Reserpine	0.29	85	0.25	41
Osmotic shock	0.07	21	0.05	8

affected by these treatments as well as by nigericin in the presence of K^+ , nigericin combined with valinomycin, the proton translocase inhibitor tributyltin, the protonophore carbonyl cyanide *p*-trifluoro-

Fig. 4. *pH dependency of 5-hydroxytryptamine uptake by isolated granules*

Incubations were performed for 20 min at 6.6 μM -5-hydroxy[^{14}C]tryptamine in 25 mM-Hepes buffer adjusted to the indicated pH values with KOH. Net uptake is expressed as the apparent intragranular content of the amine observed in the absence (O) or presence (●) of 3 mM-MgATP $^{2-}$.

methoxyphenylhydrazone and by reserpine. Neither the permeant anion I^- , nor the impermeant anion SO_4^{2-} , affected 5-hydroxytryptamine uptake in either the presence or the absence of exogenous ATP. Comparison of these data with ΔpH and $\Delta\psi$ determinations made under the same conditions (Hutton, 1982) indicated that a common feature of agents that reduced 5-hydroxytryptamine uptake was their ability to reduce the ΔpH across the granule membrane. KI, which increases the transmembrane ΔpH in the presence of ATP, did not, however, increase 5-hydroxytryptamine uptake.

The inhibition of ATP-stimulated 5-hydroxytryptamine uptake by reserpine was not accompanied by significant changes in the transmembrane ΔpH and $\Delta\psi$ (Table 1). The concentration of reserpine at which inhibitory effects on uptake were seen was of a similar magnitude to that reported to affect catecholamine uptake by isolated chromaffin granules (Phillips, 1974) but lower than that required to promote catecholamine efflux from these organelles (Phillips, 1978). The concentration of reserpine that was maximally effective here, however, was 100-fold lower than that which decreased net 5-hydroxytryptamine uptake in intact insulinoma cells.

Discussion

Cellular uptake studies

The insulinoma tissue used here as a model system to investigate B-cell 5-hydroxytryptamine transport provided an apparently homogeneous preparation of B-cells with an insulin content approx. 50% that of B-cells of normal islet tissue. The predominant biogenic amine in the insulinoma, as in rat islets, is 5-hydroxytryptamine (Feldman *et al.*, 1979). The tumour content of the amine-metabolizing enzyme monoamine oxidase is similar, whereas that of catechol *O*-methyltransferase is greater than that of rat islets (Feldman *et al.*, 1979). Reserpine inhibited 5-hydroxytryptamine uptake by these cells to a small extent, an effect that was consistent with the effects of this compound *in vivo* on the 5-hydroxytryptamine content of guinea-pig B-cell granules (Jaim-Etcheverry & Zieher, 1968).

The initial rates, the time course and the concentration-dependency of 5-hydroxytryptamine uptake by the tumour cells at 5-hydroxytryptamine concentrations above $1\mu\text{M}$ were similar to data obtained in *ob/ob* mouse islets (Hellman *et al.*, 1972; Lindstrom *et al.*, 1980). The transport process operative under these conditions appeared to be principally one of diffusion. Studies with mouse islet cells (Lindstrom, 1981) have indicated that at submicromolar 5-hydroxytryptamine concentrations there is a second Na^+ -dependent 5-hydroxytryptamine uptake process that may correspond to the high-affinity uptake system that is present in brain tissue (Ross & Renyi, 1967). There was no evidence of this, however, in our experiments. This may be related to a species difference, the fact that the cells presently used were of tumour origin or perhaps that the active-transport system resides in other cells of the islet. It must be pointed out that the uptake of 5-hydroxytryptamine either by tumour or by islet cells at submicromolar amine concentrations is at least three orders of magnitude lower than that observed in brain synaptosomes at comparable 5-hydroxytryptamine concentrations (Shaskan & Snyder, 1970).

Subcellular localization studies suggested that the tumour cells selectively incorporated radioactivity from 5-hydroxytryptamine into the granule pool. This was consistent with morphological localization of amines in islet secretory granules (see Owman *et al.*, 1973) and prompted an investigation of the transport properties of these organelles.

Studies with isolated granules

The uptake of 5-hydroxytryptamine by isolated granules under a variety of incubation conditions paralleled changes in their chemiosmotic behaviour. Previous investigations have shown that ATP-induced changes in the transmembrane ΔpH and

$\Delta\psi$ in these organelles are related to the activity of an Mg^{2+} -dependent proton-translocating ATPase within the granule membrane (Hutton, 1982; Hutton & Peshavaria, 1982). This enzyme may also be responsible for the transmembrane ΔpH that is evident in granules even without ATP present. A pH gradient formed *in vivo* could be retained after isolation of the granules by virtue of the high internal buffering capacity of the granule and the low permeability of its membrane to protons.

The resting ΔpH appeared to provide the principal driving force for 5-hydroxytryptamine uptake by granules in the absence of ATP. The constancy of the ratio of intragranular concentration of 5-hydroxytryptamine to that of the medium over a wide range of external concentrations was consistent with a process whereby the membrane was permeated by the free base form of the amine by a simple diffusion mechanism. Protonation of the base in the acidic interior of the granule would result in the accumulation of an impermeant cationic form of the amine. Such a process would reach apparent saturation at high external 5-hydroxytryptamine concentrations when accumulation of the amine exceeded the buffering capacity of the granule interior, which indeed was the case. The response of granule 5-hydroxytryptamine uptake to changes in the external pH was consistent with this mechanism since uptake increased as a function of the transmembrane ΔpH . An effect of increasing pH to increase the concentration of the free base in the medium (the pK_a of the amine group of 5-hydroxytryptamine is approx. 9.8) and hence the rate of amine uptake may have been a further factor here, where steady-state conditions had not been attained.

It can be predicted from the proposed mechanism that the ratio of the intragranular to extragranular concentration of 5-hydroxytryptamine cannot be greater than that of H^+ . In many instances, however, the gradient of 5-hydroxytryptamine radioactivity exceeded that of H^+ by a factor of two. This discrepancy would be accommodated within the above scheme if the 5-hydroxytryptamine incorporated into the granule bound to internal structures or formed a non-ideal solution within the granule interior. Control incubations performed under hypo-osmotic conditions suggested that binding did occur; there is evidence for the latter process occurring in amine-storage granules (see Njus *et al.*, 1981) but it cannot be evaluated in the present instance.

The ATP-stimulated component of 5-hydroxytryptamine uptake by granules could be distinguished from the process involved in the basal uptake in being inhibited by low concentrations of reserpine. Such uptake was apparently coupled to ATPase activity since inhibition of the enzyme by tributyltin blocked ATP-stimulated accumulation of 5-hydroxytryptamine by granules. The maintenance

of a transmembrane ΔpH appeared to be a necessary though not sufficient condition for this process to occur as evidenced by the effects on 5-hydroxytryptamine uptake of manipulation of the medium pH, or the addition of $(NH_4)_2SO_4$ or nigericin to the medium.

Chromaffin granules of the adrenal medulla and platelet dense granules maintain ΔpH gradients and $\Delta\psi$ values across their enveloping membranes of similar magnitudes to those of the insulin granule. In the former organelles amine transport occurs by the exchange of a cationic form of the amine for two protons (Johnson *et al.*, 1981; Carty *et al.*, 1981). Both the ΔpH and the $\Delta\psi$ drive amine uptake in this case and can support the formation of a gradient of amines across the membrane of approx. 1000:1 (Phillips, 1974; Johnson *et al.*, 1981; Carty *et al.*, 1981). A similar transport system could account for the effects of ATP and reserpine on 5-hydroxytryptamine uptake by insulin secretory-granule preparations. However, such a process would be at least one-tenth as active. This difference, and possibly differences in transport at the level of the plasma membrane, could account for the much lower content of amines in the insulinoma in comparison with classical amine-storage tissues.

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