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CHARACTERISTICS OF 5-HYDROXYTRYPTAMINE TRANSPORT IN PANCREATIC ISLETS

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1 Transmembrane transport of ^{3}H-labelled 5-hydroxytryptamine (5-HT) by isolated pancreatic islets of non-inbred ob/ob mice was studied.

2 5-HT was vigorously accumulated in a temperature-dependent way by the islet cells.

3 Studies of the concentration-dependence of [^{3}H]-5-HT uptake revealed complex kinetics with one component being saturated at 1 to 3 μM 5-HT (apparent association constant 0.6×10^6 M^{-1}) and the other non-saturated up to 1 mM 5-HT.

4 The saturable uptake was inhibited by Na^+-deficiency and metabolic poisoning with 2,4-dinitrophenol and antimycin A, whereas the non-saturable component was not affected.

5 Omission of K^+, Ca^{2+} or Mg^{2+} did not affect the uptake rate.

6 It is concluded that 5-HT is taken up by pancreatic β-cells by mechanisms very similar to those observed in thrombocytes and neurones.

Introduction

Although tryptaminergic mechanisms may be involved in the regulation of insulin secretion (Lernmark, 1971; Lebovitz & Feldman, 1973; Wilson, Downs Jr, Feldman & Lebovitz, 1974; de Leiva, Tanenberg, Anderson, Greenberg, Senske & Goetz, 1978), the detailed mechanisms are not well known. 5-Hydroxytryptamine (5-HT) is a potent inhibitor of glucose-induced insulin secretion (Telib, Raptis, Schröder & Pfeiffer, 1968; Feldman & Lebovitz, 1970; Gagliardino, Nierle & Pfeiffer, 1974), but 5-HT can also stimulate secretion at a substimulatory concentration of glucose (Telib, Raptis, Schröder & Pfeiffer, 1968; Gagliardino, Nierle & Pfeiffer, 1974). Previous studies on the uptake and subcellular distribution of radioactively labelled 5-HT in pancreatic islets suggested that the amine is transported into the islet cells and associated with the insulin secretory granules (Hellman, Lernmark, Sehlin & Täljedal, 1972; Mahoney & Feldman, 1977). Mechanisms for transmembrane transport of 5-HT have been characterized extensively in thrombocytes and synaptosomes. In those systems, 5-HT appears to be taken up by two different routes: one saturable route with high affinity and low capacity, and one non-saturable mechanism (Pletscher, 1968; Shaskan & Snyder, 1970; Stahl & Meltzer, 1978). We have investigated whether similar transport mechanisms operate in the pancreatic islets. Islets rich in β-cells were isolated from non-inbred ob/ob mice and their uptake of radioactive 5-HT characterized with respect to its kinetics and dependence on cellular metabolism and inorganic cations.

Methods

Animals and isolation of pancreatic islets

Adult, non-inbred obese-hyperglycaemic mice from a local colony (Umeå-ob/ob) were deprived of food overnight. The animals were killed by decapitation under ether anaesthesia and their pancreatic glands isolated by free-hand microdissection (Hellerström, 1964).

Incubations

The basal medium for isolation and incubation of the islets was a salt-balanced buffer of essentially the same composition as Krebs-Ringer bicarbonate (De Luca & Cohen, 1964), except that the bicarbonate was replaced by 20 mM Hepes (2-(*N*-hydroxyethylpiperazin-*N'*-yl)ethanesulphonic acid), pH 7.4; the medium was equilibrated with ambient air. All experiments were started by incubating batches of 2 to 4 islets for 30 min at 37°C in 1 ml non-radioactive medium. The islets were then transferred to new vials containing 200 μl of medium supplemented with 5-hydroxy-[G-^{3}H]tryptamine creatinine sulphate and [U-^{14}C]-sucrose at the concentrations given in the legends to

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Tables and Figures. Radioactive sucrose was used as an extracellular marker to correct for extracellular radioactivity (Hellman, Sehlin & Täljedal, 1971a, b). Incubations were carried out at 37°C and with shaking (140 strokes/min).

Weighing of islets and measurement of radioactivity

Incubated islets were transferred to pieces of aluminium foil, freed of surrounding fluid with a micropipette, freeze-dried overnight (−40°C, 0.1 Pa), and weighed on a quartz-fibre balance. Weighed islets were dissolved in 100 µl Hyamine and analyzed for radioactivity in a scintillation spectrometer with Instafluor as scintillation liquid. The discriminators were set such that less than 0.5% of the ^{3}H counts were also counted in the ^{14}C channel and that 25% of the ^{14}C counts were also counted in the ^{3}H channel. Corrections for this spill-over were made. The specific radioactivity of each incubation medium was determined by measuring the radioactivity of 5-µl samples of the medium.

Evaluation of results

Results are expressed as µmol of 5-HT equivalents with the same specific radioactivity as in the incubation medium. The statistical probability that the effect of an alteration of the incubation medium was due to chance was estimated from the mean difference between the test and control incubations in a series of identical but separate experiments. Both the two-tailed Student's *t* test and the two-tailed Wilcoxon's rank sum test were used and the *P*-values from both tests are given in the legends to Tables.

Chemicals

5-Hydroxy[G-^{3}H]tryptamine creatinine sulphate and [U^{14}C]-sucrose were from The Radiochemical Centre, Amersham, Bucks. Unlabelled 5-hydroxytryptamine creatinine sulphate (5-HT), antimycin A and 2-(*N*-2-hydroxyethylpiperazin-*N'*-yl)ethanesulphonic acid (Hepes) were from Sigma Chemical Co., St. Louis, Mo. U.S.A. Instafluor and *p*-(diisobutylcresoxyethoxyethyl)dimethylbenzylammonium hydroxide (Hyamine) were from Packard Instrument Co., Downers Grove, Ill., U.S.A. 2.4-Dinitrophenol was from BDH Chemicals Ltd., Poole. All other chemicals were commercially available reagents of analytical grade.

Results

Time- and temperature-dependence

Figure 1 shows the time- and temperature-dependence of the 5-HT uptake by isolated islets. At an extracellular 5-HT concentration of 0.2 or 1.5 µM, uptake proceeded for at least 2 h. Assuming an intracellular water space of 1.2 l/kg dry weight of islets (Hellman, Sehlin & Täljedal, 1971a, b), the mean intracellular concentration of labelled 5-HT after 2 h was 13.2 µM or 42.5 µM respectively. These values correspond to an accumulation of 60 or 28 times the extracellular concentration of 5-HT. The capacity of the islet cells to concentrate labelled 5-HT from the medium was markedly temperature-dependent, since lowering the temperature from 37 to 0°C decreased the uptake in 2 h by as much as 90% (0.2 µM 5-HT in medium) or 77% (1.5 µM 5-HT in medium).

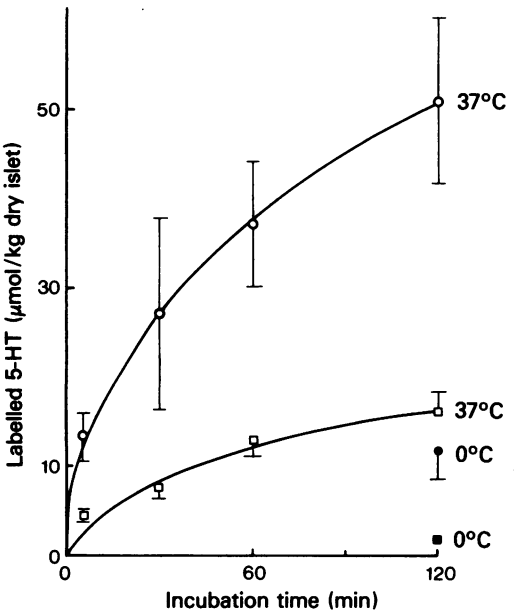

Figure 1 Time- and temperature-dependence of 5-hydroxytryptamine (5-HT) uptake. After a preliminary incubation for 30 min in basal medium, the islets were incubated for various periods of time in the same type of medium also containing 5-hydroxy[G-^{3}H]tryptamine (458 TBq/mol) and 40 µM [U-^{14}C]-sucrose (14.1 TBq/mol). Incubations were carried out at 37°C with either 0.2 µM (□) or 1.5 µM 5-HT (○). Some experiments were performed at 0 C (ice-bath) with the two 5-HT concentrations (solid symbols). Mean values for 4–6 separate experiments; vertical bars show s.e. mean.

Concentration-dependence

The concentration-dependence of 5-HT uptake at 37°C was investigated by incubating islets for 5 min or 2 h with 0.05 to 1000 µM of labelled amine (Figures 2 and 3). The short incubations were used to estimate the initial rate of uptake whereas the long

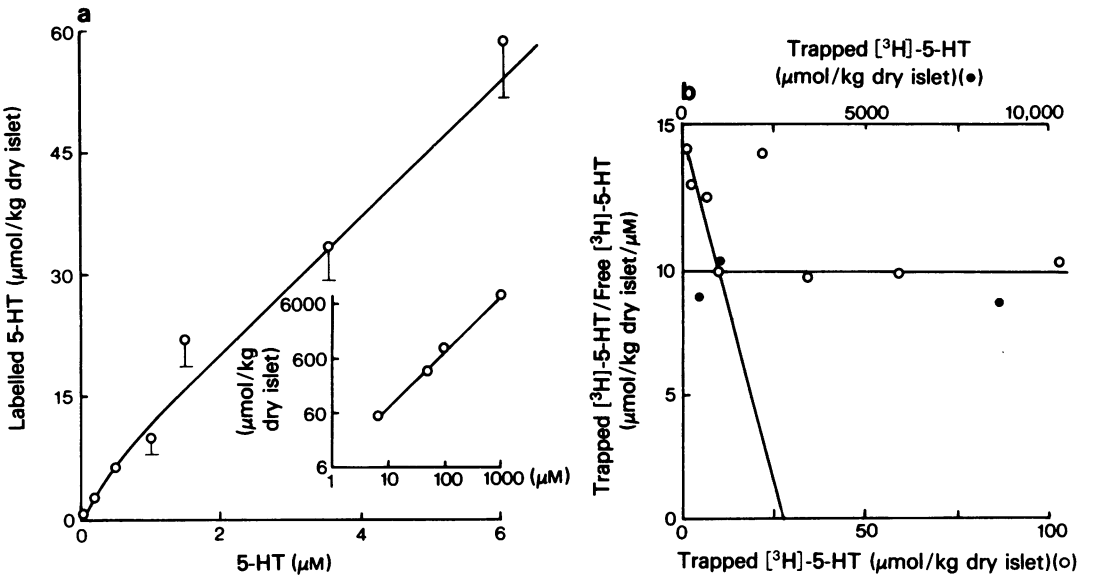

Figure 2(a) Concentration-dependence of short-term uptake of 5-hydroxytryptamine (5-HT). Islets were subjected to a preliminary incubation as in Figure 1. They were then incubated for 5 min in basal medium supplemented with 50 nM to 1 mM [^{3}H]-5-HT (458 GBq to 458 TBq/mol) and 40 μM [^{14}C]-sucrose (14.1 TBq/mol). Mean values (with bars when s.e. mean is larger than width of mean symbols) for 4 to 8 separate experiments. The inset gives the results with higher 5-HT concentrations plotted on a logarithmic scale. (b) The data in (a) are given as a Scatchard plot where the amounts of labelled 5-HT taken up (trapped) are plotted against the ratio of trapped over free label.

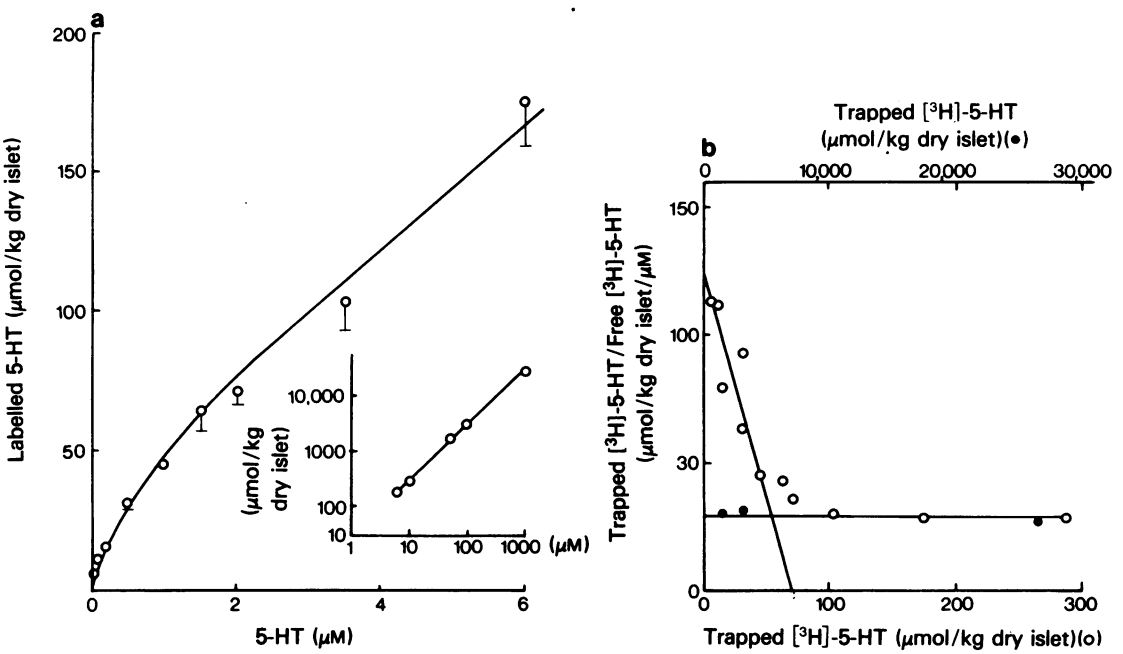

Figure 3(a) Concentration-dependence of net uptake of 5-hydroxytryptamine (5-HT). Experiments were performed as in Figure 2a except that the incubation time was 2 h. Mean values for 3–7 separate experiments; vertical lines show s.e. mean. Results are presented as in Figure 2a. (b) The data from (a) are given as a Scatchard plot.

incubations would disclose the net uptake. In both cases the uptake appeared to have complex kinetics with one component being saturated at 1 to 3 μM external 5-HT, and the other being non-saturated up to at least 1 mM 5-HT. Scatchard plots revealed that the saturable component had an apparent association constant of about 1.6×10^6 M^{-1} in 5 min incubations and about 1.8×10^6 M^{-1} in 2 h incubations.

Table 1 Effects of cations on the islet uptake of labelled 5-hydroxytryptamine (5-HT)

Omission of cations	*Uptake of labelled 5-HT (μmol/kg dry wt of islets)*
	0.2 μM 5-HT
None (control)	1.87 ± 0.45 (7)
Na^+	0.58 ± 0.22 (7)*
K^+	1.18 ± 0.19 (7)
None (control)	2.69 ± 0.93 (7)
Ca^{2+}	2.73 ± 0.46 (7)
Mg^{2+}	1.96 ± 0.46 (7)
	1.5 μM 5-HT
None (control)	24.7 ± 5.9 (9)
Na^+	20.3 ± 5.5 (9)†
K^+	27.1 ± 7.9 (9)
	25 μM 5-HT
None (control)	231.1 ± 19.7 (15)
Na^+	221.1 ± 29.8 (13)

Pancreatic islets were incubated for 5 min in basal medium supplemented with the concentrations of [^{3}H]-5-HT listed (18.3 to 458 TBq/mol) and 40 μM [^{14}C]-sucrose (14.1 TBq/mol). In the test media cations were omitted as indicated; Na^+ was replaced by choline$^+$. The ionic modifications also applied to a preliminary incubation for 30 min. Mean values ± s.e. mean for the numbers of experiments stated in parentheses. *$P < 0.05$ with Student's t test and Wilcoxon rank sum test; †$P < 0.05$ with the t test and $P < 0.1$ with the rank sum test.

Cation-dependence

Table 1 shows the effects of cations on the initial uptake of labelled 5-HT at three different extracellular concentrations. The concentrations were chosen to trace mainly the saturable high-affinity mechanism (0.2 μM), the non-saturable, low-affinity mechanism (25 μM), or a mixture of both (1.5 μM). Replacement of Na^+ with choline$^+$ led to a marked reduction of uptake at 0.2 μM, and a smaller but significant inhibition at 1.5 μM; uptake at 25 μM 5-HT was not affected by Na^+ deficiency. Omission of K^+, Ca^{2+} or Mg^{2+} had no significant effect at any concentration. None of the cations (Na^+, K^+, Ca^{2+}, or Mg^{2+}) affected the net uptake of labelled 5-HT measured in 2 h of incubation (not shown).

Metabolic blockade

To study whether the islet uptake of 5-HT depended on the cellular metabolism, the effects of 0.5 mM 2.4-dinitrophenol and 0.1 mM antimycin A were investigated (Table 2). Such metabolic poisoning of the islets reduced the 5-HT uptake at 0.2 μM 5-HT, whereas the uptake at 1.5 or 25 μM was not significantly changed.

Discussion

Previous studies on the pancreatic islets of non-inbred ob/ob mice suggested an uptake of 5-hydroxytryptamine (5-HT) into the β-cells (Hellman *et al.*, 1972; Mahoney & Feldman, 1977). Differential centrifugation of homogenates from labelled islets showed a preferential localization of radioactive 5-HT in a frac-

Table 2 Effects of 2,4-dinitrophenol and antimycin A on the islet uptake of labelled 5-hydroxytryptamine (5-HT)

5-HT (μM)	*Incubation time (min)*	*Uptake of labelled 5-HT (μmol/kg dry wt. of islets)* *Control*	
0.2	5	1.64 ± 0.04 (5)	1.38 ± 0.05 (5)*
0.2	120	8.93 ± 0.92 (8)	7.79 ± 1.15 (8)
1.5	5	16.2 ± 1.9 (6)	15.7 ± 2.6 (6)
1.5	120	46.9 ± 5.8 (5)	55.3 ± 2.1 (5)
25	5	231.1 ± 19.7 (15)	207.5 ± 28.3 (13)

Islets were incubated for 5 or 120 min in basal medium, containing the concentrations of [^{3}H]-5-HT listed (18.3 to 458 TBq/mol) and 40 μM [^{14}C]-sucrose (14.1 TBq/mol). The test media also contained 0.5 mM 2,4-dinitrophenol in combination with 0.1 mM antimycin A. These substances were also present during the preliminary incubation for 30 min. Mean values ± s.e. mean for the numbers of experiments stated in parentheses. *$P < 0.02$ with Student's t test.

tion enriched in insulin secretory granules (Hellman *et al.*, 1972). Similarly, autoradiography and electron-microscopy revealed that 5-HT, converted intracellularly from exogeneous 5-hydroxytryptophan, was stored mainly in the insulin granules of β-cells (Ekholm, Ericson & Lundquist, 1971). Gylfe (1978) showed a correlation between the rates of insulin secretion and unidirectional efflux of radioactive 5-HT from islets labelled with 5-hydroxytryptophan.

Determination of transmembrane 5-HT movements may be complicated by the metabolism of 5-HT to other compounds. The pancreatic β-cells contain monoamine oxidase activity (Feldman & Chapman, 1975), and one might expect that intracellular 5-HT is degraded to some extent. However, islets incubated with 5-hydroxytryptophan showed only very low values for the content (Gylfe *et al.*, 1973) and release (Gylfe, 1978) of 5-hydroxyindoleacetic acid, indicating a slow 5-HT metabolism. The present data, especially those obtained in brief incubations, may therefore mainly represent the islet cell content of labelled 5-HT. Pancreatic islets from ob/ob mice were used because they contain an unusually high proportion of β-cells (>90%; Hellman, 1965); thus the results are probably representative of the β-cells.

The most extensive studies on the transmembrane transport of 5-HT have been carried out with neurones and thrombocytes. These cells are generally thought to have similar uptake mechanisms (Abrams & Solomon, 1969; Sneddon, 1973). Two alternative hypotheses for 5-HT uptake have been proposed. According to the first, 5-HT transport is mediated by one high-affinity, low-capacity active transport mechanism as well as by passive diffusion (Pletscher, 1968; Stahl & Meltzer, 1978). The other hypothesis proposes that two active transport systems are working in parallel, one with high affinity and low capacity and another with low affinity and high capacity (Shaskan & Snyder, 1970). The high-affinity component is sodium-dependent (Bogdanski & Brodie, 1966; Sneddon, 1969; Shaskan & Snyder, 1970), and inhibited by metabolic blockers (Blackburn, French & Merrills, 1967; Stahl & Meltzer, 1978), whereas the low-affinity component is not.

Our present results seem best explained by the first-mentioned hypothesis. The kinetics of 5-HT uptake were complex with one component being saturated at about 1 to 3 μM extracellular 5-HT and the other component being nonsaturated up to at least 1 mM. The dependence on sodium ions and cellular metabolism was also strikingly similar to that in neurones and thrombocytes. Thus, metabolic inhibition and sodium deficiency reduced the initial uptake (5 min) of 5-HT in the presence of a low extracellular 5-HT concentration but had no, or less pronounced, effects at higher 5-HT concentrations. These results conform with our previous observation that sodium deficiency did not affect the islet uptake of 5-HT at an extracellular concentration of 5 mM (Hellman *et al.*, 1972). It therefore appears that the pancreatic β-cells are equipped with a mechanism for active transport of 5-HT operating at low concentrations of external 5-HT.

The low-affinity mechanism dominating at higher concentrations of 5-HT has the characteristics of a passive flux, since it was not affected by metabolic poisoning or sodium deficiency and was not saturated up to at least 1 mM 5-HT. However, it should be kept in mind that there was a more than 20 fold accumulation of the amine at the higher concentration of external 5-HT as well. Our previous work showed saturable kinetics in the concentration range of 5 to 25 mM 5-HT with an apparent K_m of 10 mM (Hellman *et al.*, 1972). Perhaps this saturation at very high 5-HT concentrations reflects limitations in the cellular capacity for 5-HT storage rather than properties of the membrane transport mechanism.

Low temperature reduces both the high-affinity and the low-affinity uptake of 5-HT in neurones and thrombocytes (Blackburn *et al.*, 1967; Ross & Renyi, 1974; Stahl & Meltzer, 1978). The same effect is suggested by the present data on islet cells showing that low temperature markedly reduced the capacity of the islets to accumulate labelled 5-HT. This result makes it seem unlikely that any major part of the 5-HT uptake is due to surface binding of the amine, since such binding would not be expected to be very temperature-dependent.

The fact that we have uncovered transport mechanisms operating at very low extracellular concentrations of biogenic amine emphasizes the possibility that the incorporation of amines into the β-cells is a mechanism of physiological relevance. However, since the substrate specificity of these transport routes have not been assessed in detail, it cannot be ruled out that the natural substrate is some substance other than 5-HT.

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