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FURTHER STUDIES ON 5-HYDROXYTRYPTAMINE TRANSPORT IN PANCREATIC ISLETS AND ISOLATED β-CELLS

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1 The transport mechanism for ^{3}H-labelled 5-hydroxytryptamine (5-HT) in isolated pancreatic islets of non-inbred *ob/ob* mice was further characterized.

2 Isolated β-cells accumulated 5-HT to the same degree and with the same Na^+-dependence as whole islets.

3 Imipramine inhibited the uptake in a concentration-dependent way.

4 Reserpine did not affect the uptake or efflux rates.

5 Glucose stimulation of insulin secretion did not affect the uptake rate.

6 It is concluded that the observed islet uptake of [^{3}H]-5-HT represents an intracellular accumulation by the β-cells. Mechanisms at the level of the plasma membrane may be rate-limiting for this process.

Introduction

It is thought that tryptaminergic mechanisms are involved in the secretory function of the pancreatic β-cells (Lernmark, 1971a; Lebovitz & Feldman, 1973; Wilson, Downs, Feldman & Lebovitz, 1974; De Leiva, Tanenberg, Anderson, Greenberg, Senske & Goetz, 1978; Jacoby & Bryce, 1978). It has previously been shown that external 5-hydroxytryptamine (5-HT) is taken up by *ob/ob*-mouse pancreatic islets by two different mechanisms, one with high affinity and low capacity, and the other with low affinity and high capacity (Lindström, Sehlin & Täljedal, 1980). The high-affinity mechanism was inhibited by metabolic poisoning and Na^+ deficiency and therefore fulfils some traditional criteria of an active transport. This was not observed for the low-affinity mechanism, which predominates at the high concentrations of 5-hydroxytryptamine found to affect insulin release, but also this mechanism leads to a marked accumulation of 5-HT. The uptake mechanisms may involve both regulating steps at the level of the plasma membrane and specific binding to intracellular structures. In particular it has been suggested that the insulin secretory granules accommodate large amounts of 5-hydroxytryptamine (Ekholm, Ericson & Lundquist, 1971; Hellman, Lernmark, Sehlin & Täljedal, 1972). To analyse further the different steps in β-cell uptake of 5-HT, the effects of imipramine, reserpine and insulin secretagogues have been studied. The possible involvement of non-β-cell structures in islet 5-HT uptake has been studied by use of partially purified β-cell suspensions.

Methods

After deprivation of food overnight, adult, non-inbred obese-hyperglycaemic mice (Umeå *ob/ob*) were killed by decapitation under ether anaesthesia. The pancreata were rapidly immersed in the basal medium described below and the islets isolated by free-hand microdissection (Hellerström, 1964).

Media

The medium used throughout the experiments was a salt-balanced buffer of essentially the same composition as Krebs-Ringer bicarbonate (De Luca & Cohen, 1964), except that bicarbonate was substituted for by 20 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid). The gas phase was ambient air and pH was 7.4. Unless stated otherwise in the legends to the figures and the tables, all media also contained 3 mM D-glucose. To obtain the concentrations stated in the legends to the tables, reserpine was first dissolved in dimethylsulphoxide (DMSO) and then 1 μl was added per ml of the incubation medium.

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Incubations

After a 30 min preincubation period at 37°C in 1 ml non-radioactive medium, groups of 2 – 4 islets were transferred to vials containing 200 μl of medium supplemented with 5-hydroxy [G-^{3}H] tryptamine creatinine sulphate and [U-^{14}C]-sucrose. Radioactive sucrose was used as an extracellular marker to correct for extracellular radioactivity (Hellman, Sehlin & Täljedal, 1971a,b). During the incubation the vials were kept at 37°C and shaken (140 strokes/min). 'Washing' in these experiments means that the islets were transferred to a large volume (5 ml) of non-radioactive medium and incubated for 120 min.

Weighing of islets and measurement of radioactivity

The incubations were ended by transferring the islets to small pieces of aluminium foil and removing the excess fluid with the aid of a micropipette. They were then freeze-dried overnight (−40°C; 0.1 Pa) and the dry weight was determined on a quartz-fibre balance. Weighed islets were dissolved in 100 μl Hyamine-10X and the radioactivity was determined in a liquid scintillation spectrometer with Instafluor (Packard) as the scintillation liquid. To determine the specific radioactivity of the incubation media, the radioactivity of 5 μl samples from each vial was measured. Results are given as μmol of [^{3}H]-5-HT (with the same specific radioactivity as in the medium) per kg dry weight of islets or islet cells. The dry weight of cells was calculated assuming the average dry weight of a collagenase-isolated *ob/ob*-mouse islet to be approximately 3 μg and the recovery of cells in the preparation procedure approximately 50% (Lernmark, 1974).

Studies with isolated pancreatic β-cells

The procedure for the isolation of β-cells from pancreatic islets has been described (Lernmark, 1974; Lernmark & Winblad, 1977). Briefly, about 350 islets were isolated by collagenase digestion as described by Lacy & Kostianovsky (1967) and then shaken in a Ca^{2+}-free medium supplemented with 1 mM EGTA and DNAase (5 μg/ml). The cells were then placed in a 50 mg/ml bovine serum albumin solution and centrifuged at 50 g for 5 min to remove non-cellular structures and damaged β-cells. The experimental procedures for the study of transmembrane transport into isolated β-cells has been given by Lernmark, Sehlin & Täljedal (1975). In the present study, uptake of [^{3}H]-5-HT was measured and [^{14}C]-sucrose used as a marker of the extracellular space, using the same type of HEPES-buffered medium as described under Media.

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 and *N*-2-hydroxyethylpiperazine-*N'*-2-ethane-sulphonic acid (HEPES) were bought from Sigma Chemical Co., St. Louis, Mo., U.S.A. Instafluor and *p*-(diisobutylcresoxyethoxyethyl) dimethylbenzylammonium hydroxide (Hyamine) were bought from Packard Instrument Co., Downers Grove, Ill., U.S.A. Dimethylsulphoxide was purchased from Fisher Scientific Co., Fair Lawn, NJ, U.S.A. Imipramine was a gift from ACO Läkemedel AB, Solna, Sweden, and reserpine was a gift from Ciba Geigy AG, Basel, Switzerland.

Results

Uptake of labelled 5-hydroxytryptamine by isolated β-cells

A previous study (Lindström *et al.*, 1980) showed great similarities between the characteristics of 5-HT uptake in pancreatic islets and those in thrombocytes or synaptosomes. To check whether structures, such as microvessels, connective tissue, nerve terminals or thrombocytes, contribute significantly to the islet accumulation of labelled 5-HT, the uptake of [^{3}H]-5-

Table 1 Effects of sodium deficiency on the uptake of labelled 5-hydroxytryptamine(5-HT) in isolated β-cells

	Uptake of labelled 5-HT (μmol/kg dry weight of cells)	
	0.2 μM	100 μM
Control	3.09 ± 0.53 (6)	1522.9 ± 236.5 (9)
Without sodium	2.49 ± 0.40**(6)	1329.2 ± 224.2*(9)

Suspensions of isolated pancreatic islet cells were incubated for 5 min in basal medium supplemented with 0.2 or 100 μM[^{3}H]-5-HT (6.9 – 458 TBq/mol) and 40 μM[^{14}C]-sucrose (14.1 TBq/mol). In the test medium, Na^+ was replaced by choline. This modification also applied to the 30 min preliminary incubation period. Mean values ± s.e. mean for the numbers of experiments stated in parentheses. $^{**}P<0.02$; $^{*}P<0.05$ with Student's *t* test.

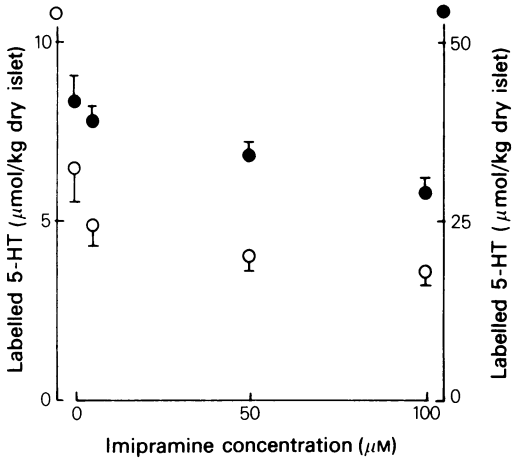

Figure 1 Effect of imipramine on the uptake of labelled 5-hydroxytryptamine(5-HT) by pancreatic islets. Groups of 2 – 4 islets were incubated for 120 min with 0.2 μM (○) or 1.5 μM (●) [^{3}H]-5-HT (458 TBq/mol) present. [^{14}C]-sucrose 40 μM (14.1 TBq/mol) was used as a marker for the extracellular space. Mean for 6 – 8 experiments; vertical lines show s.e. mean.

HT by a suspension of β-cells as described by Lernmark (1974) was measured. The purity of the β-cell preparation has not been ascertained. However, it is probable that most of the nerve terminals and thrombocytes, the non-endocrine constituents most likely to accumulate 5-HT, were removed during the isolation procedure. The cells were spun down to a pellet through a 5% albumin gradient at 50 *g* for 5 min. To obtain a crude preparation of thrombocytes centrifugation at 100 – 200 *g* for 15 – 20 min is necessary (see Murphy, Colbum, Davis & Bunney, 1969), while to obtain an enriched fraction of nerve endings usually even higher speeds are necessary (see Franke & Uebelhack, 1978). Nerve terminals capable of accumulating 5-HT are also normally rare in mice (Falck & Hellman, 1963, Cegrell, 1968). Table 1 shows values for the labelled 5-HT (0.2 or 100 μM) uptake by suspended β-cells calculated as mmol/kg dry weight of cells. The magnitude of the uptake at 0.2 μM or 100 μM external 5-HT was close to the corresponding values for intact islets, reported here or previously (Lindström, *et al.*, 1980). The inhibition by Na^+ deficiency of the uptake at the low 5-HT concentration and the smaller, if any, effect at the higher concentration also fit those previous data. Since most non-β-cell structures capable of accumulating amines are probably removed during the preparation procedure, the results support the idea that the 5-HT uptake observed in intact islets is representative of the β-cells.

Inhibition of 5-hydroxytryptamine uptake by imipramine

The tricyclic antidepressant agent, imipramine, is known to block transmembrane fluxes of 5-HT in neurones (Carlsson, Corrodi, Fuxe & Hökfelt, 1969) and thrombocytes (Pletscher, 1968). Figure 1 shows that imipramine inhibited net uptake of [^{3}H]-5-HT by pancreatic islets in a concentration-dependent manner. The relative magnitude of this inhibition was about the same whether a very low (0.2 μM) or a higher (1.5 μM) concentration of labelled 5-HT was used. Imipramine (50 μM) also inhibited the initial (5 min) uptake of 0.2 μM or 1.5 μM labelled 5-HT, provided that imipramine was present also during a preliminary incubation for 30 min (Table 2). These observations support our previous suggestion that the islet uptake of labelled 5-HT mainly represents cellular transmembrane uptake (Lindström *et al.*, 1980).

Lack of effects of reserpine on 5-hydroxytryptamine uptake

Several previous observations have suggested that 5-HT is accumulated by the insulin storage granules in the β-cells (Jaim-Etcheverry & Zieher, 1968;

Table 2 Effect of imipramine on the uptake of ^{3}H-labelled 5-hydroxytryptamine (5-HT) by pancreatic islets

Imipramine (μM)	*5-HT* (μM)	*Uptake of labelled 5-HT* (μmol/kg dry weight of islet)
0	0.2	0.86 ± 0.08 (8)
50	0.2	0.64 ± 0.07 (8)*
0	1.5	10.92 ± 1.67 (8)
50	1.5	6.87 ± 1.02 (8)*

Islets were incubated for 5 min in basal medium supplemented with 0.2 μM (458 TBq/mol) or 1.5 μM (458 TBq/mol) [^{3}H]-5-HT and 40 μM [^{14}C]-sucrose (14.1 TBq/mol) and with or without imipramine at the concentration given. In the test groups imipramine was also present during the 30 min preliminary incubation period. Mean ± s.e.mean for the number of experiments given. * $P < 0.05$ with Student's *t* test.

Table 3 Effect of reserpine on islet uptake of labelled 5-hydroxytryptamine (5-HT)

Reserpine (μM)	*Incubation time* (min)	*5-HT* (μM)	*Uptake of labelled 5-HT* (μmol/kg dry weight of islet)
0	5	0.2	2.08 ± 0.12 (7)
5	5	0.2	2.85 ± 0.78 (6)
0	120	0.2	7.68 ± 0.86 (4)
5	120	0.2	8.38 ± 3.72 (4)
0	5	1.5	18.27 ± 1.46 (9)
5	5	1.5	17.35 ± 0.89 (9)
0	120	1.5	43.36 ± 3.72 (8)
5	120	1.5	40.15 ± 4.67 (8)

Groups of two to four islets were incubated in basal medium supplemented with 0.2 μM (458 TBq/mol) or 1.5 μM (458 TBq/mol) [^{3}H]-5-HT. Reserpine was dissolved in dimethylsulphoxide to give the concentrations shown when added as 1 μl per ml. Mean ± s.e.mean for the number of experiments given.

Ekholm *et al.*, 1971; Hellman *et al.*, 1972; Gylfe, 1978). Reserpine is an established inhibitor of vesicular uptake of 5-HT in thrombocytes (Reimers, Allen, Cazenave, Feuerstein & Mustard, 1977) and neurones (Slotkin, Seidler, Whitmore, Lau, Salvaggio & Kirksey, 1978). We therefore studied the effect of reserpine on islet uptake of labelled 5-HT, in order to test the idea of a vesicular accumulation of 5-HT in the β-cells. Table 3 shows that reserpine (5 μM) had no significant effect on the islet uptake of labelled 5-HT, whether 0.2 μM or 1.5 μM 5-HT was used. Reserpine at 50 μM also had no effect in equivalent experiments. However, at this concentration reserpine was not kept in solution during the whole incubation period. Reserpine had no effect on the rate of efflux of labelled 5-HT from islets prelabelled with 1.5 μM [^{3}H]-5-HT. Also pretreatment of the islets for 120 min with 50 μM reserpine did not affect their ability to accumulate 5-HT.

Insulin secretagogues do not affect 5-hydroxytryptamine uptake

From studies on leucocytes it was suggested (Woodin & Wieneke, 1970), that exocytotic secretion may take place without breakage of the fused secretory granule membrane and plasma membrane. During membrane fusion, external material may enter the granule sac and thus be incorporated within intracellular vesicles. It has also been demonstrated, by ultracytochemical techniques, that stimulation of insulin secretion by glucose increases the incorporation of peroxidase in a vesicular pool in the β-cells (Orci, Malaisse-Lagae, Ravazzola, Amherdt & Renold, 1973). Against this background, we tested the effect of glucose and theophylline on the islet uptake of labelled 5-HT; glucose being an effective secretagogue of insulin release from β-cells of *ob/ob*-mice (Lernmark, 1971b) and theophylline a potentiator of that release (Idahl, 1973). Figure 2 shows that 20 mM D-glucose and 5 mM theophylline had no effect on the uptake of 1.5 μM 5-HT, either when the incubation time was 5 min (apparent initial uptake) or 120 min (net uptake). It therefore seems unlikely, that the low affinity uptake of 5-HT takes place through a direct vesicular capture coupled to exocytosis as described above.

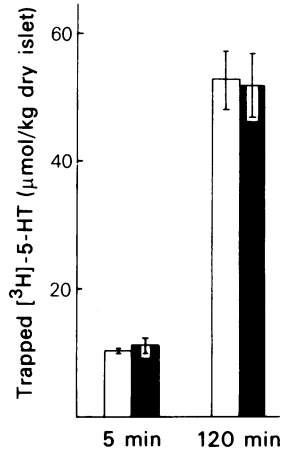

Figure 2 Effect of glucose and theophylline on the uptake of labelled 5-hydroxytryptamine(5-HT) by pancreatic islets. 5-HT concentration 1.5 μM. The amount of [^{3}H]-5-HT accumulated by the islets given as mean (vertical lines show s.e. mean) for the controls (open columns) or when 20 mM D-glucose and 5 mM theophylline was added (solid columns).

Discussion

Many species, including the *ob/ob*-mouse (Hansen & Hedeskov, 1977), contain endogenous 5-HT in their pancreatic islets. The islets also readily accumulate

5-HT, either directly from exogenous 5-HT (Hellman *et al.*, 1972; Mahoney & Feldman, 1977; Lindström *et al.*, 1980) or through the uptake of 5-hydroxytryptophan (5-HTP) (Ekholm, *et al.*, 1971; Tjälve, 1971), which is rapidly converted intracellularly to 5-HT (Gylfe, Hellman, Sehlin & Täljedal, 1973).

Islet cells probably do not hydroxylate tryptophan, as in isolated islets 5-HT was formed from 5-HTP but not from L-tryptophan (Gylfe *et al.*, 1973). Also, in rats, no 5-HT fluorescence was found in the islets cells after high oral doses of L-tryptophan (Gagliardino, Zieher, Iturriza, Hernández & Rodriguez, 1971). Since, moreover, 5-HTP normally occurs at very low concentrations, at least in human blood plasma (Engbaek & Magnussen, 1978), it is doubtful whether 5-HT is normally synthesized in the mammalian islets although they show decarboxylase activity (Gylfe *et al.*, 1973). An understanding of the detailed mechanisms for the uptake and storage of 5-HT may therefore be of fundamental importance. Lindström *et al.*, (1980) showed that *ob/ob*-mouse islets accumulate exogenous 5-HT *in vitro* by at least two mechanisms both leading to a vast intracellular accumulation of the amine. One mechanism, predominating at very low 5-HT concentrations showed features of an active transport. The present study was designated primarily to characterize further the other, low-affinity mechanism, preponderant at the high concentrations of 5-HT known to influence insulin secretion *in vitro*.

The first finding was that an isolated and partially purified β-cell suspension accumulated 5-HT to the same extent and with about the same sodium dependence as whole islets. This strongly indicates that the islet uptake is mainly confined to the β-cells. It also indicates, indirectly, that 5 min is sufficient time for the amine to equilibrate in the extracellular space of intact islets.

Lowering the temperature has a drastic inhibitory effect on islet uptake and efflux rates of 5-HT (Lindström *et al.*, 1980). This would not be the case if any major part of the amine taken up by the β-cells was bound to the outer cell surface and thus suggests that external 5-HT crosses the β-cell plasma membrane. Imipramine inhibited the 5-HT uptake in a concentration-dependent way at both low and high 5-HT concentrations. If imipramine acts by the same mechanism in pancreatic islets as in thrombocytes and neurones (Pletscher, 1968; Carlsson *et al.*, 1969), these results provide further support for the idea that both the high affinity and the low affinity 5-HT uptake mechanisms lead to an intracellular accumulation of the amine.

To test the hypothesis that the accumulation of 5-HT is due to vesicular binding with characteristics similar to those found in thrombocytes and neurones, reserpine was used. Reserpine did not affect either the uptake or efflux rates of 5-HT in pancreatic islets *in vitro*. This is in keeping with the observations by Hellman *et al.* (1972) and Aleyassine & Sin Hang Lee (1972). However, contradictory microscopic (Falck & Hellman, 1964; Jaim-Etcheverry & Zieher, 1968; Ekholm *et al.*, 1971) and fluorometric (Lundquist, Sundler, Håkanson, Larsson & Heding, 1975) studies have also been presented. The lack of effect by reserpine suggests a difference in 5-HT uptake mechanisms by pancreatic β-cells as compared with thrombocytes or neurones.

The present *in vitro* results with reserpine prompted a search for a possible alternative route for vesicular accumulation of 5-HT. Results obtained by Loewenstein (1966) suggested that fusion areas between biological membranes may be highly permeable due to escape of Ca^{2+}. Woodin & Wieneke (1970) suggested that secretion in leucocytes might occur at such a fusion between the plasma membrane and the secretory granule vesicle without breakage of the membranes. It is not known whether such a mechanism operates in the pancreatic β-cells. However, it has been found that stimulation of insulin secretion by D-glucose results in a β-cell uptake of electron dense peroxidase confined to a vesicular intracellular pool (Orci, Malaisse-Lagae, Ravazzola, Amherdt & Renold, 1973). In the present study, islets were challenged with 20 mM D-glucose and 5 mM theophylline to stimulate insulin secretion strongly. This treatment did not influence islet uptake of labelled 5-HT and it is therefore no indication that external 5-HT is taken up by vesicular capture coupled to insulin discharge. The results conform with previous work showing no effect of 20 mM D-glucose on initial uptake of labelled 5 mM 5-HT (Hellman *et al.*, 1972).

In summary, the results presented in this paper strongly suggest that both the high affinity and the low affinity uptake of 5-HT by pancreatic islets are confined to the β-cells. It also gives further evidence that both mechanisms result in an intracellular accumulation of 5-HT; this accumulation is insensitive to reserpine and insulin secretagogues.

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