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Uptake Mechanisms of 5-HT in Pancreatic β-cells

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INTRODUCTION

Tryptaminergic mechanisms are thought to be involved in the secretory function of the pancreatic β-cells (4,7-9,20). Studies on the uptake and subcellular distribution of radioactively labelled 5-hydroxytryptamine (5-HT) in pancreatic islets suggested that the amine is transported into the islet cells and associated with the insulin secretory granules (6,13). The present experiments were undertaken to further characterize the 5-HT uptake; I have investigated whether transport mechanisms for 5-HT similar to those in thrombocytes and synaptosomes (14,16,19) operate in the pancreatic islets. Islets rich in β-cells were isolated from non-inbred ob/ob mice, and their uptake of labelled 5-HT was characterized with respect to its kinetics and dependence on cellular metabolism and inorganic cations. To further analyze the different steps in β-cell uptake of 5-HT, the effects of imipramine and reserpine have been studied. The possible involvement of non-β-cell structures in islet 5-HT uptake has been studied by using partially purified β-cell suspensions.

METHODS

Adult, non-inbred obese-hyperglycemic mice (ob/ob) were starved overnight, killed by rapid decapitation and their pancreatic islets isolated by free hand microdissection (5). The medium used throughout was a Hepes-buffered Krebs-Ringer solution, pH 7.4, equilibrated with ambient air. Unless differently stated, all media contained 3 mM D-glucose. Reserpine was first dissolved in dimethylsulfoxide and then taken 1 µl per 1 ml into the incubation medium to get the concentration stated in the legends. Temperature was kept at 37°C unless stated otherwise.

After a 30 min preliminary incubation, 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 various test substances. (U-^{14}C)sucrose was added as a marker for the extracellular space. After the incubation period

islets were freeze-dried overnight, weighed on a quartz-fibre balance and analyzed for radioactivity in a scintillation spectrometer. Results are expressed as μmol of 5-HT equivalents with the same specific radioactivity as in the incubation medium. The procedures for isolating β-cells from pancreatic islets (10,12) and for studying transmembrane transport into isolated β-cells (11) have been described.

RESULTS AND DISCUSSION

Figure 1 shows that at an extracellular 5-HT concentration of 0.2 or 1.5 μM, uptake proceeded for at least 2 h. The values correspond to an accumulation of 60 or 28 times the extracellular concentration of 5-HT. The ability to concentrate labelled 5-HT from the medium was markedly temperature-dependent. This 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.

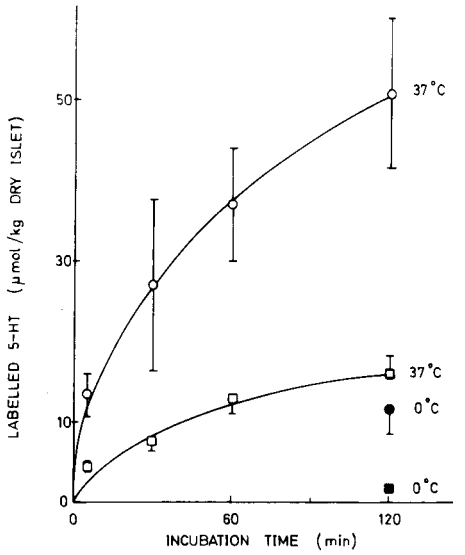

Figure 1 Time-and temperature-dependence of 5-hydroxytryptamine (5-HT) uptake. Islets were incubated for various periods of time in medium containing 5-hydroxy(G-^{3}H)-tryptamine (458 TBq/mol) and 40 μM (U-^{14}C)-sucrose (14.1 TBq/mol). Incubations were carried out at 37°C with either 0.2 μM (□) or 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 SEM.

Figure 2(a) shows the concentration-dependent uptake of 5-HT during the first 5 min - i.e. the apparent initial uptake. The Scatchard plot of the same data (Fig 2b) shows that the uptake mechanisms are complex. There is one component with high affinity for 5-HT which is saturated at around 1-3 μM extracellular amine and one component with lower affinity which is not saturated up to at least 1 mM. The apparent association constant for the saturable component was about 1.6×10^{6} M^{-1}.

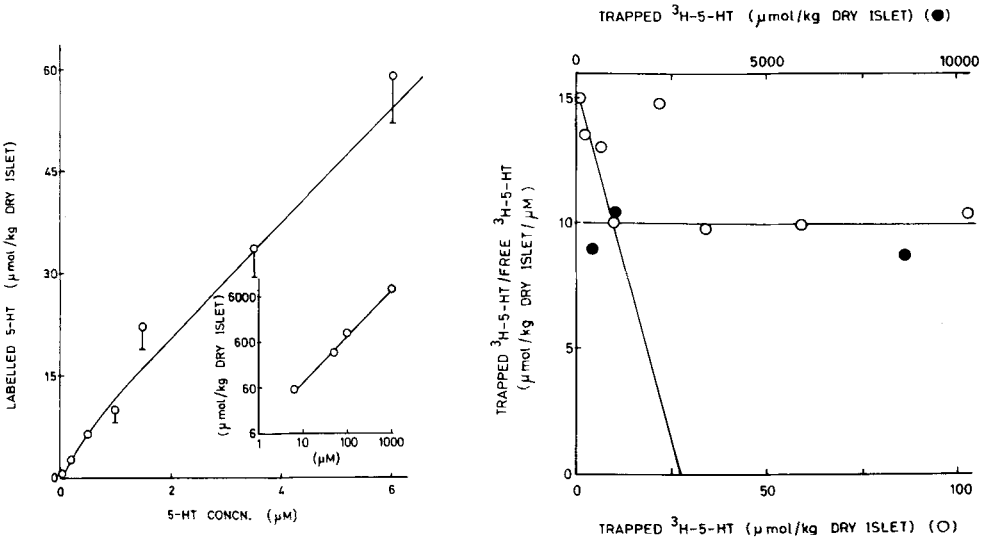

Figure 2(a) Concentration-dependence of short-term uptake of 5-hydroxytryptamine (5-HT). Islets were incubated for 5 min in basal medium supplemented with 50 nM to 1 mM (^{3}H)-5-HT (458 TBq/mol) and 40 μM (^{14}C)-sucrose (14.1 TBq/mol). Mean values (with bars when SEM is larger than width of mean symbols) for 4 to 8 separate experiments. The inset gives the results with higher 5-HT concentrations plotted on a logarithmic scale. (b) The data (a) are given as a Scatchard plot.

Table 1 shows that at low concentrations of 5-HT, when the high-affinity mechanism is preponderant, the uptake is sodium dependent. The low affinity mechanism shows no such sodium dependence. It can also be seen that omission of potassium, calcium or magnesium does not affect initial uptake rates. Blocking the metabolism (Table 2) affects the uptake only at low 5-HT concentrations. Thus, only the high-affinity uptake mechanism is clearly dependent on cellular metabolism.

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 ±SEM for the numbers of experiments stated in parentheses. *P<0.05.

Table 2 Effects of metabolic blockade 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 ± SEM for the numbers of experiments stated in parentheses. *P<0.02.

The uptake of 5-HT by the β-cells shows complex kinetics with one high-affinity/low-capacity mechanism having some features of an active transport, and one low-affinity/high-capacity mechanism lacking these features. However, also the low-affinity mechanism gave an accumulation of 5-HT of more than 20 times the extracellular concentration. These findings conform well with the uptake of 5-HT in thrombocytes and neurones (1,2,16,18,19). The finding (Table 3) that imipramine inhibited initial uptake is also in agreement (3,14).

Table 3 Imipramine inhibits the uptake of ^{3}H-labelled 5-HT by pancreatic islets

Imipramine (μM)	5-HT (μM)	Uptake of labelled 5-HT (μmol/kg dry wt of islets)
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 present also during a 30 min preliminary incubation period. Mean ± SEM for the number of experiments given. *P<0.05.

However, there are also differences between pancreatic β-cells on the one hand (Table 4) and thrombocytes or neurones on the other (15,17), since reserpine had no effect whatsoever on the islet uptake of labelled 5-HT.

An isolated and partially purified β-cell suspension accumulated 5-HT to the same degree and with the same sodium dependence as whole islets (Table 5). This strongly indicates that the islet uptake is mainly confined to the β-cells.

The fact that we have uncovered transport mechanism of 5-HT operating at very low extracellular concentrations of biogenic amine emphasizes the possibility that the incorporation of amines into the β-cell is a mechanism of physiological relevance.

Table 4 Reserpine has no effect on islet uptake of labelled 5-HT

Reserpine concentration (μM)	Incubation time (min)	5-HT concentration (μM)	Uptake of labelled 5-HT (μmol/kg dry wt of islets)
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. Mean ± SEM for the number of experiments given.

Table 5 Sodium deficiency lowers the uptake of labelled 5-HT in isolated beta-cells

Uptake of labelled 5-HT (μmol/kg dry wt of cells)		
	0.2 μM	100 μM
Control	3.09±0.53(6)	1522.9±236.5(9)
Omission of 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 ± SEM for the numbers of experiments stated in parenthesis. **P<0.02. *P<0.05.

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REFERENCES

1. Blackburn, K.J., French, P.C. & Merrills, R.J.: 5-Hydroxytryptamine uptake by rat brain in vitro. Life Sci (Oxford) 6:1653-1663, 1967.
2. Bogdanski, D.F. & Brodie, B.B.: Role of sodium and potassium ions in storage of norepinephrine by sympathetic nerve endings. Life Sci (Oxford) 5:1563-1569, 1966.
3. Carlsson, A., Corrodi, H., Fuxe, K. & Hökfelt, T.: Effect of antidepressant drugs on the depletion of intraneuronal brain 5-hydroxytryptamine stores caused by 4-methyl-α-ethyl-meta-tyramine. Eur J Pharmacol 5:357-366,1969.
4. De Leiva, A., Tanenberg, R.J., Anderson, G., Greenberg, B., Senske, B. & Goetz, F.C.: Serotoninergic activation and inhibition: effects on carbohydrate tolerance and plasma insulin and glucagon. Metabolism 27:511-520, 1978.
5. Hellerström, C.: A method for the microdissection of intact pancreatic islets of mammals. Acta Endocrinol (Copenh.) 45:122-132, 1964.
6. Hellman, B., Lernmark, Å., Sehlin, J. & Täljedal, I.-B.: Transport and storage of 5-hydroxytryptamine in pancreatic β-cells. Biochem Pharmac 21:695-706, 1972.
7. Jacoby, J.H. & Bryce, G.F.: Monoaminergic modulation of pancreatic endocrine secretion. Gen Pharmac 9:411-419, 1978.
8. Lebovitz, H.E. & Feldman, J.M.: Pancreatic biogenic amines and insulin secretion in health and disease. Fedn Proc 32:1797-1802, 1973.
9. Lernmark, Å.: The significance of 5-hydroxytryptamine for insulin secretion in the mouse. Horm Metab Res 3:305-309, 1971.
10. Lernmark, Å.: The preparation of, and studies on, free cell suspensions from mouse pancreatic islets. Diabetologia 10:431-438, 1974.
11. Lernmark, Å., Sehlin, J. & Täljedal, I.-B.: The use of dispersed pancreatic islet cells in measurements of transmembrane transport. Anal Biochem 63: 73-79, 1975.
12. Lernmark, Å. & Winblad, B.: Scanning electron microscopy of surface changes on dispersed pancreatic β-cells following stimulation of insulin release. Med Biol 55:141-147, 1977.
13. Mahoney, C. & Feldman, J.M.: Species variation in pancreatic islet monoamine uptake and action. Diabetes 26:257-261, 1977.
14. Pletscher A.: Metabolism, transfer and storage of 5-hydroxytryptamine in blood platelets. Br J Pharmac Chemother 32:1-16, 1968.
15. Reimers, H.-J., Allen, D.J., Cazenave, J.-P., Feuerstein, I.A. & Mustard, J.F.: Serotonin transport and storage in rabbit blood platelets - the effects of reserpine and imipramine. Biochem Pharmac 26:1645-1655, 1977.
16. Shaskan, E.G. & Snyder, S.H.: Kinetics of serotonin accumulation into slices from rat brain: relationship to catecholamine uptake. J Pharmac exp Ther 175:404-418, 1970.
17. Slotkin, T.A., Seidler, F.J., Whitmore, W.L., Lau, C., Salvaggio, M. & Kirksey, D.F.: Rat brain synaptic vesicles: Uptake specificities of (^{3}H)-norepinephrine and (^{3}H)serotonin in preparations from whole brain and brain regions. J Neurochem 31:961-968, 1978.
18. Sneddon, J.M.: Sodium-dependent accumulation of 5-hydroxytryptamine by rat blood platelets. Br J Pharmac 37:680-688, 1969.
19. Stahl, S.M. & Meltzer, H.Y.: A kinetic and pharmacologic analysis of 5-hydroxytryptamine transport by human platelets and platelet storage granules: comparison with central serotonergic neurons. J Pharmac exp Ther 205:118-132, 1978.
20. Wilson, J.P., Downs Jr., R.W., Feldman, J.M. & Lebovitz, H.E.: Beta cell monoamines: further evidence for their role in modulating insulin secretion. Am J Physiol 227:305-312, 1974.