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Opposite Effects of 5-Hydroxytryptophan and 5-Hydroxytryptamine on the Function of Microdissected *ob/ob*-Mouse Pancreatic Islets

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Summary. The effects of 5-hydroxytryptamine and 5-hydroxytryptophan on insulin release and $^{45}Ca^{2+}$ uptake in islets microdissected from *ob/ob* mice were studied. At a concentration of 4 mmol/l both compounds slightly stimulated insulin release at a low glucose concentration (3 mmol/l). Insulin release induced by 20 mmol/l D-glucose was inhibited by 4 mmol/l 5-hydroxytryptamine but potentiated by 4 mmol/l L-5-hydroxytryptophan. Mannoheptulose (20 mmol/l) blocked the combined effects of 20 mmol/l D-glucose and 4 mmol/l L-5-hydroxytryptophan on insulin release. $^{45}Ca^{2+}$ uptake was inhibited by 4 mmol/l 5-hydroxytryptamine and stimulated by 4 mmol/l L-5-hydroxytryptophan. Mannoheptulose (20 mmol/l) did not affect the $^{45}Ca^{2+}$ uptake induced by the latter. When 4 mmol/l L-5-hydroxytryptophan was present only during the 30-min preincubation period, 20 mmol/l-glucose-induced insulin release and $^{45}Ca^{2+}$ uptake during a subsequent incubation period were inhibited. Externally added 5-hydroxytryptamine (4 mmol/l) did not change the effects of 4 mmol/l L-5-hydroxytryptophan on insulin release and $^{45}Ca^{2+}$ uptake. It is concluded that, when added directly into the incubation medium, 5-hydroxytryptophan has effects on insulin release and $^{45}Ca^{2+}$ uptake which are opposite to those observed when 5-hydroxytryptamine is added. These effects do not seem to be mediated by 5-hydroxytryptamine formed intracellularly from 5-hydroxytryptophan.

Key words: Pancreatic islets, *ob/ob* mouse, insulin release, calcium uptake, 5-hydroxytryptophan, 5-hydroxytryptamine.

The role of 5-hydroxytryptamine (5-HT) in the B cell has been studied intensely during the last two decades. 5-HT has been suggested to be involved in normal insulin secretion as well as in the development of diabetes mellitus [1–5] but the cellular mechanisms for its action in B cells are not well understood. Most data presented support the concept that 5-HT inhibits glucose-induced insulin release, while having a slight stimulatory effect on basal insulin release [for review 6–11].

Islet cells can accumulate and store 5-HT intracellularly [12–14] and the effects of elevation of intracellular 5-HT have been studied. Such elevation can be achieved in two ways; the B cells can take up and effectively decarboxylate the precursor, 5-hydroxytryptophan (5-HTP) [12–14] or they can accumulate exogenously added 5-HT [15–18]. The 5-HT accumulated is only slowly metabolized by the islets [14]. In studies using the first method, it has generally been assumed that 5-HTP itself is inert and that the effects observed are due to the 5-HT formed. However, there are some indications that when added directly into the incubation medium, 5-HTP does not exert the same inhibitory effect on glucose-induced insulin release as 5-HT [19–21].

We have aimed at elucidating whether 5-HTP affects islet cells solely by being metabolized to 5-HT or whether it has independent effects on the islet secretory function. Thus we have measured the actions of 5-HTP or 5-HT or combinations of both on insulin release and uptake of $^{45}Ca^{2+}$ by B cell-rich islets of *ob/ob* mice. $^{45}Ca^{2+}$ uptake was measured because insulin secretagogues stimulate Ca^{2+} uptake into B cells and this process is believed to be an important link in the B cell stimulus-secretion coupling [22].

Materials and Methods

Chemicals

$^{45}Ca^{2+}$ was from Amersham International, Bucks., UK. ^{125}I-insulin was obtained from Hoechst, Frankfurt/Main, FRG. 5-Hydroxytryptamine creatinine sulphate, DL-5-hydroxytryptophan, creatinine sulphate, L-5-hydroxytryptophan, 2-(*N*-hydroxyethylpiperazine-*N'*-yl)ethanesulphonic acid (Hepes), and mannoheptulose were from Sigma Chemicals, St. Louis, Missouri, USA. Bovine serum albumin was from Miles Laboratories, Stoke Poges, Bucks., UK, and crystalline mouse insulin was kindly prepared by Novo, Copenhagen, Denmark. Instafluor and *p*-(diisobutyl-cresoxyethoxyethyl)-dimethylben-

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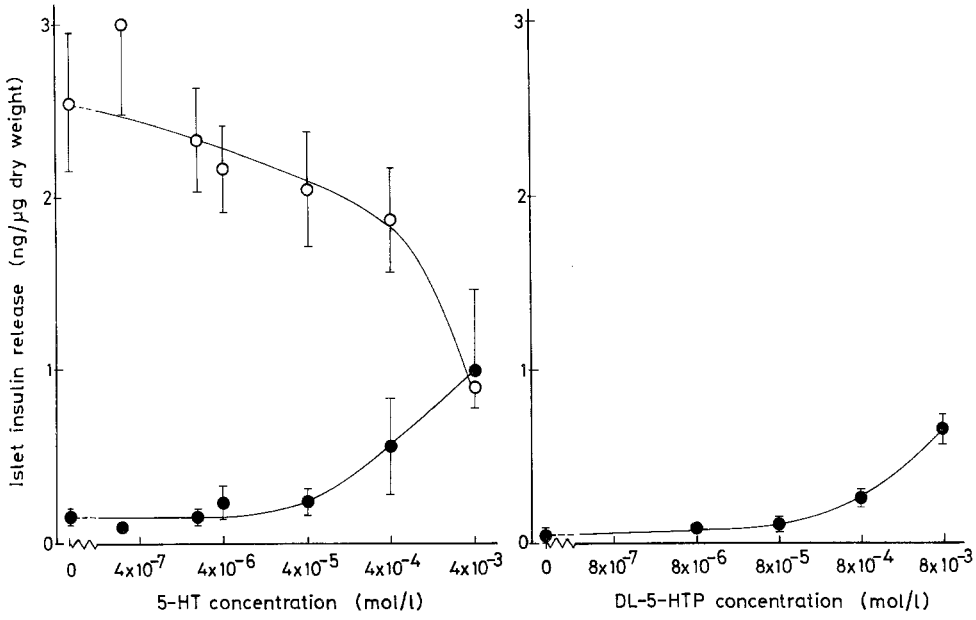

Fig. 1. Pancreatic islets were incubated for 60 min in Krebs-Ringer bicarbonate medium supplemented with 3 mmol/l D-glucose (●) or 20 mmol/l D-glucose (○) and increasing concentrations of 5-HT (left panel) or DL-5-HTP (right panel). The same concentrations of 5-HT or 5-HTP were also present in the basal medium (3 mmol/l D-glucose) during av 30-min preliminary incubation. The symbols denote mean ± SEM (with bars when SEM is larger than width of mean symbol) for seven to eight experiments

zylammonium hydroxide (Hyamine) were bought from Packard, Downers Grove, Illinois, USA. Other chemicals were commercially available reagents of analytical grade.

Animals and Isolation of Pancreatic Islets

Adult, non-inbred obese-hyperglycaemic mice from a local colony (Umeå *ob/ob*) were starved overnight and killed by decapitation under ether anaesthesia. Their pancreatic islets were isolated by free-hand microdissection [23]. These islets contain an unusually high proportion of B cells (>90%; [24]), which respond to stimulators and inhibitors of insulin release [25].

Incubation Media

Experiments on insulin secretion were performed with Krebs-Ringer bicarbonate medium [26] equilibrated with O_2 + CO_2 (95%:5%), pH 7.4. The basal medium in $^{45}Ca^{2+}$ uptake studies had the same composition as Krebs-Ringer bicarbonate, except that the bicarbonate was replaced by 20 mmol/l Hepes (2-(*N*-hydroxyethylpiperazine-*N'*-yl) ethanesulphonic acid), pH 7.4. This medium was equilibrated with ambient air. In the $LaCl_3$ washing medium (see below), phosphate and sulphate were replaced by chloride and the medium was buffered with 5 mmol/l Tris-HCl to pH 7.4 [27]. All basal media contained D-glucose (3 mmol/l). 5-HT was added in the form of a creatinine sulphate complex. Control experiments showed no effect of 4 mmol/l creatinine sulphate alone on 20 mmol/l-glucose-induced insulin release or $^{45}Ca^{2+}$ uptake at 3 or 20 mmol/l glucose (data not shown).

$^{45}Ca^{2+}$ Uptake

After a preliminary incubation for 30 min, groups of four to five islets were incubated for 120 min with $^{45}Ca^{2+}$ (2.56 mmol/l, 0.3 TBq/mol) at 37 °C. The islets were transferred to Tris-buffered medium (5 ml) containing $LaCl_3$ (2 mmol/l) and incubated for 60 min at 37 °C. The rationale for this procedure has been described previously [27]. The uptake measured during 120 min represents the net uptake at apparent isotope equilibrium [27].

Insulin Release

After pre-incubation for 30 min in basal medium, groups of two to three islets were incubated for 60 min in 300 µl medium at 37 °C. These media contained bovine serum albumin (1 mg/ml) and test substance as indicated in the legends to figures and tables. Insulin released into the incubation medium was measured radioimmunologically using crystalline mouse insulin as a reference.

Weighing of Islets and Counting of Radioactivity

Incubated islets were transferred to pieces of aluminium foil and freed of surrounding fluid with a micropipette. They were then freeze-dried overnight (−40 °C, 0.1 Pa) and weighed on a quartz-fibre balance. In $^{45}Ca^{2+}$ experiments, weighed islets were dissolved in Hyamine (100 µl) and their radioactivity was counted in a scintillation spectrometer with Instafluor as the scintillation liquid. The specific radioactivity of each incubation medium was also determined.

Evaluation of Results

$^{45}Ca^{2+}$ uptake is expressed as µmol of Ca^{2+} with the same specific radioactivity as in the incubation medium, and insulin release as ng of insulin released into the medium. A two-tailed paired Student's t-test was used to compare the difference between the test and control incubation in a series of identical but separate experiments.

Results

Effects of 5-HT and 5-HTP on Insulin Release

Figure 1 shows that 5-HT at 0.4–4 mmol/l stimulated basal (3 mmol/l glucose) insulin release but suppressed 20 mmol/l-D-glucose-stimulated insulin release. At 4 mmol/l 5-HT, only 30% of the D-glucose-induced insulin release remained. Figure 1 also shows that 5-HTP stimulated basal (3 mmol/l glucose) insulin release. The effect of 4 mmol/l L-5-HTP (0.70 ± 0.15 ng insulin/µg dry weight, Table 1) is not significantly different from the effect of 8 mmol/l DL-5-HTP (0.64 ± 0.09 ng insulin/µg dry weight, Fig. 1). In contrast to 5-HT, 4 mmol/l but not lower concentrations of L-5-HTP potentiated 20 mmol/l-D-glucose-induced insulin release (Table 1).

Effects of 5-HT or 5-HTP on $^{45}Ca^{2+}$ Uptake

As shown in Table 2, 4 mmol/l 5-HT inhibited $^{45}Ca^{2+}$ uptake both at 3 mmol/l and at 20 mmol/l D-glucose. No effect was seen at lower concentrations (data not shown).

L-5-HTP (4 mmol/l) strongly increased $^{45}Ca^{2+}$ uptake at 3 mmol/l D-glucose but did not increase uptake above the level induced by 20 mmol/l glucose alone (Table 3). DL-5-HTP (8 mmol/l) had the same stimulatory effect on $^{45}Ca^{2+}$ uptake as L-5-HTP (4 mmol/l) (data not shown). Table 3 also shows that 20 mmol/l D-mannoheptulose, which blocks insulin release [25], inhibited glucose-induced $^{45}Ca^{2+}$ uptake. However, D-mannoheptulose did not affect the capacity of 4 mmol/l L-5-HTP (Table 3) or 8 mmol/l DL-5-HTP (data not shown) alone or of 4 mmol/l L-5-HTP in combination with 20 mmol/l D-glucose to stimulate $^{45}Ca^{2+}$ uptake.

Effect of Mannoheptulose on 5-HTP-Potentiated Insulin Release

Since mannoheptulose had no effect on L-5-HTP-induced $^{45}Ca^{2+}$ uptake, it was of interest to test whether this sugar could influence 5-HTP-induced insulin release. Table 3 shows that 20 mmol/l mannoheptulose inhibited the combined effect of 4 mmol/l L-5-HTP and 20 mmol/l D-glucose on insulin release. The 5-HTP-induced insulin release at 3 mmol/l D-glucose was not affected by mannoheptulose.

Effects of Pretreatment with L-5-HTP on Glucose-Induced Insulin Release and $^{45}Ca^{2+}$ Uptake

L-5-HTP is taken up by the B cells of *ob/ob* mice and converted to 5-HT, which is then stored intracellularly [14, 18]. As shown in Table 4, islets exposed to 4 mmol/l L-5-HTP only during the 30-min preincubation released less insulin and accumulated less $^{45}Ca^{2+}$ when challenged with 20 mmol/l D-glucose during the subsequent incubation period, as compared with islets preincubated without 5-HTP. Thus, the effects of 5-HTP when added to the incubation medium (Fig. 1, Tables 1 and 3) are different from those obtained when 5-HTP is present only during the preincubation period.

Effects of 5-HT on 5-HTP-Induced $^{45}Ca^{2+}$ Uptake and on 5-HTP-Potentiation of Glucose-Induced Insulin Release

Since 5-HT inhibited (Fig. 1) and L-5-HTP potentiated (Table 1) glucose-induced insulin release, it was tested whether the L-5-HTP-induced effects could be counteracted by added 5-HT. Table 5 shows that 4 mmol/l 5-HT had no effect on L-5-HTP-induced uptake of $^{45}Ca^{2+}$ nor on the L-5-HTP potentiation of glucose-induced insulin release.

Table 1. Effect of L-5-hydroxytryptophan on insulin release in mouse islets

D-glucose concentration (mmol/l)	Test substance	Insulin release (ng/μg dry weight of islets)
3	None (control)	0.22 ± 0.04 (25)
3	4 mmol/l L-5-HTP	0.70 ± 0.15 (8)[a]
20	None (control)	2.33 ± 0.29 (16)
20	0.04 mmol/l L-5-HTP	2.53 ± 0.35 (16)
20	0.4 mmol/l L-5-HTP	3.30 ± 0.46 (8)
20	4 mmol/l L-5-HTP	8.28 ± 0.82 (16)[b]

[a] $p < 0.02$ and [b] $p < 0.01$ compared with control
Batches of three islets were incubated for 60 min in basal medium supplemented with D-glucose and L-5-HTP as indicated. L-5-HTP was also included in the 30-min preincubation with 3 mmol/l D-glucose. Data are expressed as mean ± SEM for the number of experiments shown in parentheses

Table 2. Effect of 5-hydroxytryptamine on uptake of labelled Ca^{2+} in mouse islets

Test substance	Content of labelled Ca^{2+} (μmol/g dry weight of islets)
3 mmol/l D-glucose	
None (control)	10.87 ± 1.65 (7)
4 mmol/l 5-HT	7.04 ± 1.33 (7)[a]
20 mmol/l D-glucose	
None (control)	15.67 ± 0.90 (20)
4 mmol/l 5-HT	13.49 ± 1.01 (20)[b]

[a] $p < 0.05$ and [b] $p < 0.02$ compared with respective controls
After a 30-min preincubation with 3 mmol/l D-glucose, the islets were incubated for 120 min with $^{45}Ca^{2+}$, D-glucose and 5-HT and then washed with 2 mmol/l La^{3+} and the radioactivity determined. Values denoting islet content of labelled Ca^{2+} are expressed as mean ± SEM for the number of experiments given in parentheses.

Table 3. Effect of L-5-hydroxytryptophan and mannoheptulose on islet uptake of labelled Ca^{2+} and insulin release in mouse islets

Test substance	Content of labelled Ca^{2+} (μmol/g dry weight of islets)	Insulin release (ng/μg dry weight of islets)
3 mmol/l D-glucose		
None (control)	8.54 ± 0.64 (12)	0.17 ± 0.03 (15)
4 mmol/l L-5-HTP	19.77 ± 2.27 (6)[a]	0.60 ± 0.10 (16)[b]
4 mmol/l L-5-HTP + 20 mmol/l mannoheptulose	18.07 ± 2.66 (6)[a]	0.56 ± 0.07 (8)[b]
20 mmol/l D-glucose		
None (control)	15.48 ± 1.08 (12)	2.98 ± 0.37 (8)
20 mmol/l mannoheptulose	9.83 ± 1.54 (6)[a]	0.25 ± 0.05 (8)[b]
4 mmol/l L-5-HTP	15.46 ± 2.21 (6)	8.26 ± 0.61 (8)[b]
4 mmol/l L-5-HTP + 20 mmol/l mannoheptulose	15.73 ± 1.56 (6)	1.11 ± 0.19 (8)[c]

[a] $p < 0.01$ and [b] $p < 0.001$ compared with the respective controls
[c] $p < 0.001$ compared with 8.26 ± 0.61
After a 30-min preincubation with 3 mmol/l D-glucose, the islets were incubated with $^{45}Ca^{2+}$, D-glucose, L-5-HTP and mannoheptulose as indicated. Results are expressed as in Tables 1 and 2

Table 4. Effects of pretreatment with L-5-hydroxytryptophan on subsequent glucose stimulation of insulin release and uptake of labelled Ca^{2+} in mouse islets

Additives to basal medium		Insulin release (ng/μg dry weight of islets)	Content of labelled Ca^{2+} (μmol/g dry weight of islets)
Preincubation period	Incubation period		
3 mmol/l D-glucose	3 mmol/l D-glucose	0.21 ± 0.04 (7)	9.53 ± 0.96 (6)
3 mmol/l D-glucose	20 mmol/l D-glucose	2.98 ± 0.37 (8)	16.43 ± 1.73 (6)
3 mmol/l D-glucose + 4 mmol/l L-5-HTP	20 mmol/l D-glucose	0.87 ± 0.20 (8)	11.29 ± 1.13 (6)

[a] $p < 0.01$ and [b] $p < 0.001$

Islets were preincubated for 30 min with 3 mmol/l D-glucose and then incubated for 60 min (insulin release) or 120 min ($^{45}Ca^{2+}$ uptake). Glucose and L-5-HTP were added as indicated. Data are expressed as mean ± SEM for the number of experiments shown in parentheses

Table 5. Effects of 5-hydroxytryptamine on 5-hydroxytryptophan-induced uptake of labelled Ca^{2+} and 5-hydroxytryptophan-potentiation of glucose-induced insulin release in mouse islets

Glucose concentration (mmol/l)	Test substances	Insulin release (ng/μg dry weight of islets)	Content of labelled Ca^{2+} (μmol/g dry weight of islets)
3	None	0.25 ± 0.08 (8)	7.49 ± 0.27 (7)
3	4 mmol/l L-5-HTP		14.01 ± 0.90 (7)
3	4 mmol/l L-5-HTP + 4 mmol/l 5-HT		13.67 ± 0.40 (7)
20	None	2.55 ± 0.55 (8)	
20	4 mmol/l L-5-HTP	7.78 ± 1.36 (8)	
20	4 mmol/l L-5-HTP + 4 mmol/l 5-HT	8.88 ± 1.74 (8)	

[a] $p < 0.01$ and [b] $p < 0.001$

Islets were preincubated for 30 min with 3 mmol/l D-glucose and then incubated for 60 min (insulin release) or 120 min ($^{45}Ca^{2+}$ uptake). Glucose, L-5-HTP and 5-HT were added as indicated. $^{45}Ca^{2+}$ uptake was determined as in Table 2 and the values are expressed as mean ± SEM for the number of experiments shown in parentheses

Discussion

Externally added 5-HT is incorporated into the B cells of *ob/ob* mice [15–18]. The precursor, 5-HTP, is taken up and rapidly converted to 5-HT [12–14]. If 5-HTP acted exclusively via formation of 5-HT one would expect both compounds to have the same type of effect on islet functions. In contrast, this study shows that, glucose-induced insulin release was reduced by 5-HT but strongly amplified by 5-HTP. The inhibition by 5-HT is consistent with a number of previous reports showing that 5-HT (0.1–5 mmol/l) inhibits glucose-induced insulin release in vitro [3, 19, 28–33]. Also in accordance with previous in vitro [3, 21, 30, 31, 34, 35] and in vivo [31, 36–39] observations on 5-HT and 5-HTP, both these agents slightly stimulated basal insulin release. The underlying mechanisms for this effect are unknown.

The difference in effects of 5-HT and 5-HTP on glucose-induced insulin release is probably not explained by differential transmembrane distribution of 5-HT in the B cells, because externally added 5-HT did not inhibit the stimulatory effect of 5-HTP on insulin release and $^{45}Ca^{2+}$ uptake. When 5-HTP was present only during a preincubation period, in a subsequent incubation the glucose-induced insulin release was decreased. This is in accord with previous observations [35] and suggests that 5-HT formed from 5-HTP has the same inhibiting effect as 5-HT taken up as such.

Ca^{2+} uptake is involved in the regulation of insulin secretion, probably as a distal link in stimulus-secretion coupling [22]. By determining the effects of 5-HT and 5-HTP on the islet cell $^{45}Ca^{2+}$ uptake, we attempted to analyze whether they act in the stimulus-secretion coupling. As shown here this appears to be the case. 5-HT reduced the uptake of $^{45}Ca^{2+}$ and 5-HTP strongly enhanced it. Other experiments indicate that 5-HT also inhibits the glucose-effects on a number of early steps in the B cell stimulus-secretion coupling, i.e. $^{86}Rb^{+}$ efflux (K^{+} permeability) [40], $^{36}Cl^{-}$ efflux (Cl^{-} permeability) [41], phosphate flush [42], whereas 5-HTP does not affect these processes. From this we suggest that 5-HT lowers glucose-induced insulin release by reducing early steps in the stimulus-secretion coupling, whereas 5-HTP stimulates glucose-induced insulin release by activating a distal part of the coupling sequence, including Ca^{2+}. Since 5-HT does not completely block insulin release at a high glucose concentration (Fig. 1), a 5-HTP-activation of such a distal amplification mechanism may be effective despite production of 5-HT from the precursor. That mannoheptulose, a known blocker of glucose-induced insulin release [25] (Table 3), inhibited the combined effects of 5-HTP and 20 mmol/l glucose on insulin release conforms with the idea that 5-HTP acts as a potentiator of the glucose-induced secretory signal.

In agreement with previous work [43], mannoheptulose totally blocked glucose-induced $^{45}Ca^{2+}$ uptake in the La^{3+}-nondisplaceable Ca^{2+} pool. However, it had no effect on $^{45}Ca^{2+}$ uptake in the presence of both 5-HTP and 20 mmol/l glucose. One explanation for this finding could be that 5-HTP and D-glucose stimulate $^{45}Ca^{2+}$ uptake partly via separate mechanisms leading to saturable accumulation in a common compartment. The 5-HTP stimulated mechanism, being unresponsive

to mannoheptulose, would allow for maximum $^{45}Ca^{2+}$ accumulation also in the simultaneous presence of D-glucose, 5-HTP, and mannoheptulose. It is notable that, with these three compounds present, there was a dissociation between insulin release and $^{45}Ca^{2+}$ uptake into the La^{3+}-nondisplaceable pool. Although there is a striking correlation between these two phenomena in most studies [27, 43], it has been reported that dissociation between them might occur [44]. It appears likely that the Ca^{2+} mechanisms involved in insulin release are complicated and not completely represented by $^{45}Ca^{2+}$ uptake as measured here. The apparent dissociation between insulin release and $^{45}Ca^{2+}$ uptake may be elucidated as the cellular mechanisms for 5-HTP-potentiation of glucose-induced insulin release are further investigated.

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