Inhibition of Glucagon Release by Serotonin in Mouse Pancreatic Islets

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Summary. In this work we have investigated the effect of serotonin on glucagon release in mouse pancreatic islets isolated by the collagenase technique.

Incubation of the islets with serotonin (4×10^{-3} mol/l) was associated with an inhibition of glucagon output both in the basal medium (3.3 mmol/l glucose) and in the presence of arginine (10 mmol/l). The inhibitory effect of serotonin on basal glucagon release was also apparent at concentrations of 2×10^{-3} mol/l, 10^{-3} mol/l and 5×10^{-4} mol/l. Addition of 5-hydroxytryptophan (4×10^{-3} mol/l) to the incubation medium was without effect on basal glucagon output while it significantly reduced arginine-induced glucagon release. In contrast, tryptophan (4×10^{-3} mol/l) provoked glucagon secretion. As inferred from our previous human studies, the present data indicate that serotonin is able to inhibit glucagon secretion. These findings provide further support for the participation of a serotonergic mechanism in the control of A-cell function.

Key words: Mouse pancreatic islets, glucagon, insulin, serotonin, 5-hydroxytryptophan, tryptophan, creatinine sulphate.

In man, treatment with serotonin antagonists (cyproheptadine and methysergide) as well as with an inhibitor of serotonin synthesis (para-chlorophenylalanine) induces a potentiation of glucagon secretion. Because of these findings, we have suggested that serotonin modulates A-cell function by acting as an inhibitor [1]. To investigate this hypothesis further, in this work we have examined the effects of serotonin and its immediate precursor

(5-hydroxytryptophan) on glucagon release in a preparation of isolated mouse pancreatic islets. In addition, insulin output was measured.

Materials and Methods

The preparation of pancreatic islets employed has been described in detail elsewhere [2]. Briefly, four-week old male mice, fasted for 14 h, were used as pancreas donors. The islets were isolated by the collagenase technique [3], as modified by Coll-García and Gill [4]. Batches of 10 islets were incubated for 60 to 120 minutes in 2 ml of Krebs-Henseleit solution [5] (gas phase 95:5, CO₂:O₂), containing 0.2 g/100 ml bovine albumin (Cohn fraction V), 2000 U of kallikrein-trypsin inhibitor (Trasylol), and 3.3 mmol/l glucose. Conventionally, this solution will be referred to as "basal medium" throughout the text. In some experiments, arginine hydrochloride (10 mmol/l) was used as the glucagon secretagogue. Serotonin creatinine sulphate, 5-hydroxy-L-tryptophan, L-tryptophan and creatinine sulphate were added as described in the figures and text. All incubations were performed in a water bath at 37 °C, set at 60 strokes per minute.

Glucagon and insulin were measured by radioimmunoassay [6, 7]. Results are expressed as means $\pm$ SEM. N indicates the number of independent experiments. The term experiment refers to a set of incubations performed at the same time with islets prepared from a pool of eight pancreases. Within each experiment, all factors were tested simultaneously employing from 6 to 10 incubation vials. Differences between values were tested for significance by Student's t-test.

Collagenase was purchased from Boehringer Mannheim GmbH; arginine hydrochloride, seroto-

Fig. 1. Effect of serotonin on basal and arginine-induced glucagon and insulin release by mouse pancreatic islets. (Means $\pm$ SEM. N = number of independent experiments)

nin creatinine sulphate, 5-hydroxy-L-tryptophan, L-tryptophan and creatinine sulphate were obtained from Sigma Chem. Co. Glucagon- I^{125} and insulin- I^{125} were kindly supplied by Dr. I. Valverde. Glucagon antiserum (30K) was a generous gift from Dr. R.H. Unger.

Results

As shown in Figure 1, incubation of the islets with serotonin (4×10^{-3} mol/l) was associated with an inhibition of glucagon release, both in the basal medium and in the presence of arginine ($\approx 25\%$, $p < 0.03$ and $p < 0.001$, respectively). Addition of serotonin also produced a decrease in insulin secretion ($\approx 49\%$, $p < 0.01$ in the basal medium; $\approx 62\%$, $p < 0.001$ in the presence of arginine).

Since serotonin was employed as a creatinine sulphate complex, we tested the effect of creatinine sulphate in our preparation. Addition of this com-

Fig. 2. Glucagon and insulin release by mouse pancreatic islets in the presence of different concentrations of serotonin in the basal medium (Means $\pm$ SEM. N = number of independent experiments). The large dots represent statistically significant differences from the 0 serotonin value

pound to the basal medium at 4×10^{-3} mol/l failed to modify significantly glucagon and insulin secretion, although a greater output of both hormones was observed (glucagon: 186 ± 27 vs. 335 ± 68 pg/10 islets/60 min, N = 4; insulin: 2.6 ± 0.8 vs. 3.7 ± 1.6 ng/10 islets/60 min, N = 4).

Figure 2 shows that serotonin, at lower concentrations than that used in the first series of experiments, also diminished basal glucagon and insulin release. This inhibition showed a dose dependent pattern. At 5×10^{-4} mol/l, the lowest molarity tested, the reduction of glucagon secretion was still statistically significant ($p < 0.01$), while 10^{-3} mol/l was the minimal concentration of serotonin required to inhibit significantly insulin output ($p = 0.03$).

The presence of 5-hydroxytryptophan (the immediate precursor of serotonin) in the medium (Fig. 3), did not affect basal glucagon release; however, it caused a clear decrease in arginine-induced glucagon secretion ($\approx 34\%$, $p < 0.0001$). 5-Hydroxytryptophan also inhibited arginine-stimulated

Fig. 3. Effect of 5-hydroxytryptophan on basal and arginine-induced glucagon and insulin release by mouse pancreatic islets (Means $\pm$ SEM. N = number of independent experiments)

insulin release ($\approx 26\%$, $p < 0.001$); a small but significant decrease of basal insulin output ($\approx 17\%$, $p < 0.05$) was found.

Finally, tryptophan (the essential amino acid from which serotonin is derived) at 4×10^{-3} mol/l provoked glucagon secretion (292 ± 41 vs. 591 ± 118 pg/10 islets/60 min, $N = 9$, $p < 0.05$).

Discussion

The foregoing data indicate that serotonin behaves as an effective inhibitor of glucagon release in mouse pancreatic islets. This action cannot be attributed to the presence of creatinine sulphate in the serotonin complex tested since, in our preparation, this substance fails to modify significantly hormone output.

It is obvious that the concentrations of serotonin employed in our incubations can hardly be applied to physiological conditions. However, they are well

within the range described for inhibition of insulin release by the pancreas of several species [8, 9, 10].

5-Hydroxytryptophan did not affect glucagon release when added to the basal medium, although it significantly reduced arginine-stimulated glucagon output. Since mouse pancreatic A-cells are able to store serotonin after 5-hydroxytryptophan administration [11], the inhibition of glucagon secretion by this amino acid could be attributed to its conversion to serotonin; nevertheless, a direct effect cannot be excluded. It should be mentioned that exposure of the islets to 5-hydroxytryptophan was maintained for 120 minutes since, as reported by Gylfe et al. [12], the uptake of this substance by pancreatic islets of obese mice reaches a steady state after one h of incubation. In contrast, tryptophan displayed glucagonotropic activity, which is in accordance with the stimulation of glucagon secretion induced by this amino acid in dog [13] and in man [14]. On the other hand, no conversion of tryptophan to 5-hydroxytryptophan or to serotonin was found by Gylfe and associates in their "in vitro" preparation [12].

Hence, the present results lend support to the inhibitory action of serotonin on A-cell secretion, as inferred from our previous human studies [1].

Concerning insulin release, our findings confirm the inhibitory effect of serotonin described by Feldman et al. in pieces of hamster, rabbit and mouse pancreas [8, 9, 10] and extend the observations of these authors to another B-cell stimulus; i.e., arginine. The reduction of arginine-stimulated insulin release observed in the presence of 5-hydroxytryptophan, is in good agreement with previous "in vitro" and "in vivo" data obtained in rabbits and mice [15, 16, 17]. As in the case of its effect on A-cell function, the conversion of this substance to serotonin within the B-cell [12] seems to be a plausible explanation for its inhibitory action on insulin secretion.

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