Effects of Serotonin and its Antagonists on the First Phase of Insulin Release in Normal and Hypersomatotropic Rats*

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Summary

The in situ perfused rat pancreas of normal and hypersomatotropic rats was used to study the effects of serotonin and its antagonists on the release of insulin in the presence of 4.4 mM and 16.6 mM glucose. In normal rats, serotonin (1.0 x 10^{-4}M and 2.3 x 10^{-4}M) inhibited significantly the glucose-mediated first phase of insulin release but did not affect the basal release of insulin (4.4 mM glucose). The serotonin antagonists methysergide maleate (MSM) (1.0 x 10^{-4}M), Squibb (SQ) 10,631 (SQ (1.0 x 10^{-4}M) and cyproheptadine (Cy) (2.8 x 10^{-9}M) blocked the inhibitory action of serotonin. Only MSM potentiated the glucose-mediated first phase of insulin release in the presence of non-stimulatory glucose concentration (4.4 mM).

In hypersomatotropic rats, serotonin inhibited the first phase of glucose-mediated insulin release, but a 20-times higher concentration than that used in normal rats was necessary to obtain the same degree of inhibition. In contrast the antagonists MSM, SQ and Cy did not block the inhibition induced by serotonin. MSM and SQ, however, significantly stimulated insulin release in the presence of 4.4 mM glucose. The results obtained suggest that:

a) exogenous and intracellular serotonin might regulate the release of insulin by acting on the β cell membrane;
b) β cells from hypersomatotropic rats have a decreased sensitivity to serotonin, and possibly to serotonin antagonists.

Key-Words: Insulin Release – Serotonin – Serotonin Antagonists – β Cell Membrane – Hypersomatotropism – MtT-W15 Tumor

Introduction

Investigations to date on a possible role of serotonin (5-HT) on the mechanism of insulin release is still a matter of discussion. Indeed, it has been reported that 5-HT inhibits the glucose-mediated insulin release in the golden hamster (Feldman and Lebovitz 1970a; Feldman and Lebovitz 1970b), stimulates the release of insulin in rabbits (Telih, Raptis, Schröder and Pfeiffer 1968), rats (Gagliardino, Zieher, Iturria, Hernández and Rodríguez 1971) and mice (Gagliardino, Nierle and Pfeiffer 1974) while others found that in mice 5-HT inhibits (Lundquist, Ekholm and Ericson 1971) or showed no effect (Feldman and Lebovitz 1970b) upon insulin release.

Evidence has accumulated suggesting that 5-HT may play an important role modulating the release of insulin. The serotonin antagonists methysergide maleate (MSM), cyproheptadine (Cy) and cinaserin were shown to potentiate the in vitro glucose-mediated insulin
release in the golden hamster (Feldman, Quickel Jr. and Lebovitz 1972). MSM appeared to improve the overall insulin release in response to glucose challenge in maturity onset diabetic patients (Quickel Jr., Feldman and Lebovitz 1971). Recently, Feldman, Plonk, Bivens and Lebovitz (1975) reported that the impaired insulin release observed in patients with the carcinoid syndrome was mainly due to the high circulating serotonin levels. In these patients, the early peak of insulin release appeared blunted (Feldman, Marecek, Quickel Jr. and Lebovitz 1972). Except for this latter work and that of Lundquist, Ekholm and Ericson (1971) none of the above mentioned studies were carried out in models where biphasic insulin release could be observed.

It is the purpose of this work to study the effects of serotonin and its antagonists, MSM, SQ and Cy, on the basal (4.4 mM glucose) and glucose-mediated (16.6 mM glucose) first phase of insulin release from an in situ perfused rat pancreas.

The effect of serotonin and its antagonists was also studied in rats rendered hypersomatotropic by transplanting the growth hormone (GH) secreting tumor MtT-W15. Åkerblom, Martin, Garay and Moscarello (1972) have shown that 4-5 weeks after tumor implantation these rats have a 6 and 50 fold increase in insulin and GH levels respectively, but glucose levels were significantly lower (12%). In addition, the β cells of these rats have an increased sensitivity to glucose (Martin and Åkerblom 1968) and responded to continuous glucose challenge with the typical biphasic pattern of insulin release (de Bold, Bencosme and Martin 1976). These latter authors also noted that the levels of insulin obtained were higher than those seen in normal rats.

Material and Methods

Analytical Methods

The effects of serotonin and its antagonists on the first phase of IRI release were expressed as the integrated areas under the insulin curve from 3 to 8 minutes (S ARI). Statistical comparison of the results was made using the Student’s t-test. Differences were considered to be significant for p < 0.05.

Results

Effect of Serotonin and its Antagonists on Normal Rats

In a glucose concentration in the perfusate from 4.4 mM to 16.6 mM was accompanied by a considerable increase in IRI release from the pancreas (Fig. 1). A typical glucose-mediated first phase of IRI release was obtained. Serotonin at 1.0 x 10⁻⁵ M and 2.3 x 10⁻⁴ M induced a significant suppression of this glucose-mediated IRI release (Fig. 1, Table 1) but did not significantly affect basal insulin release (Fig. 2, Table 1). Inhibition obtained with 1.0 x 10⁻⁵ M serotonin was greater than that seen with 2.3 x 10⁻⁴ M serotonin, this unexpected finding is not possible to interpret at present without a detailed dose-response study. The serotonin antagonists, MSM (1.0 x 10⁻⁵ M) and Cy (2.8 x 10⁻⁹ M) effectively blocked the inhibition induced by serotonin (2.3 x 10⁻⁴ M) whereas a partial blocking effect was found with SQ (1.0 x 10⁻⁴ M) (Fig. 3, Table 1). In the presence of serotonin, only MSM significantly (p < 0.01) potentiated the glucose-mediated first phase of IRI (Fig. 3, Table 1). In the absence of serotonin, MSM significantly
Table 1. Effect of serotonin and its antagonists on the first phase of insulin release from the in situ perfused normal rat pancreas*

<table>
<thead>
<tr>
<th>Additions to perfusate</th>
<th>Insulin release in μU</th>
<th>Glucose 16.6 mM</th>
<th>4.4 mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>961.2 ± 138.6</td>
<td>64.7 ± 20</td>
<td></td>
</tr>
<tr>
<td>Serotonin (1.0 x 10⁻⁵M)</td>
<td>237.5 ± 29.9</td>
<td>P &lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>Serotonin (2.3 x 10⁻⁴M)</td>
<td>367.4 ± 81.7</td>
<td>P &lt; 0.01</td>
<td></td>
</tr>
<tr>
<td>Serotonin (2.3 x 10⁻⁴M) + MSM (1.0 x 10⁻⁴M)</td>
<td>1986.7 ± 229.1</td>
<td>P &lt; 0.01</td>
<td></td>
</tr>
<tr>
<td>Serotonin (2.3 x 10⁻⁴M) + SQ (1.0 x 10⁻⁴M)</td>
<td>611.5 ± 101.2</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Serotonin (2.3 x 10⁻⁴M) + Cy (2.8 x 10⁻⁹M)</td>
<td>877.5 ± 170.4</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>MSM (1.0 x 10⁻⁴M)</td>
<td>2766.9 ± 203.3</td>
<td>P &lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>SQ (1.0 x 10⁻⁴M)</td>
<td>985.7 ± 175.9</td>
<td>292.8 ± 39.6</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Cy (2.8 x 10⁻⁹M)</td>
<td>890.4 ± 69.7</td>
<td>60.0 ± 23.0</td>
<td>NS</td>
</tr>
</tbody>
</table>

* Mean ± SEM of six experiments
** P values refer to the significance of different experiments with respect to the presence of 16.6 mM glucose
*** P values refer to the significance of different experiments with respect to the presence of 4.4 mM glucose

Fig. 1 + 2.
Fig. 1 Glucose-mediated (■) first phase of IRI release and its inhibition by serotonin 1.0 x 10⁻⁵M (□-□) and 2.3 x 10⁻⁴M (○-○). Basal IRI release obtained for 3 minutes prior to drug or glucose administration (■). Results are expressed as the mean ± SEM of six experiments.
Fig. 2. Serotonin at 1.0 x 10⁻⁵M (□-□) and 2.3 x 10⁻⁴M (○-○) failed to modify the basal IRI release (■). Results expressed as in Figure 1.

(p < 0.001) enhanced the first phase of IRI release (Fig. 4, Table 1). In contrast, SQ and Cy did not significantly potentiate the glucose-mediated IRI release (Fig. 4, Table 1). When the serotonin antagonists were tested at non-stimulatory glucose concentration (4.4 mM) there was a significant release of IRI by MSM and SQ (Fig. 5, Table 1). In both instances there was an immediate increase in insulin release followed by a rapid decrease. The IRI in the perfusate, however, remained above the basal levels until the end of the experimental period. Cy did not stimulate the basal IRI release.

Discussion
In the present study, it has been clearly demonstrated that exogenous serotonin inhibits the first phase of insulin release from the in situ perfused pancreas of normal rats. This inhibition is effectively blocked by the serotonin antagonists MSM, SQ and Cy. Blunting of the early peak of insulin release observed in patients with the carcinoid syndrome and its return to normal levels after lowering the circulating serotonin concentration by tumor therapy (Feldman et al. 1972) is consistent with these results.

The rapidity with which serotonin and its antagonists modify the first phase of insulin release strongly suggests that both of these substances might be acting at the level of the β cell membrane since studies with islets of Langerhans (Cegrell 1968) indicate poor penetration of serotonin across “intact” cell membranes. This study reinforces the hypothesis of Feldman and Lebovitz (1972) whereby exogenous serotonin may be acting at the cell membrane.
Effects of Serotonin and its Antagonists on the First Phase of Insulin Release in Normal Rats

Table 2. Effect of serotonin and its antagonists on the first phase of insulin release from the in situ perfused tumor-bearing rat pancreas

<table>
<thead>
<tr>
<th>Additions to perfusate</th>
<th>Insulin release in μU</th>
<th>Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>16.6 mM</td>
<td>p**</td>
</tr>
<tr>
<td>None</td>
<td>5759.9 ± 529.4</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td>Serotonin (1.0 x 10^{-5}M)</td>
<td>4127.4 ± 396.7</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>Serotonin (2.3 x 10^{-5}M)</td>
<td>3009.7 ± 412.5</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>Serotonin (4.6 x 10^{-3}M)</td>
<td>2458.7 ± 280.7</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Serotonin (2.3 x 10^{-3}M) + MSM (1.0 x 10^{-4}M)</td>
<td>1427.9 ± 81.1</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Serotonin (2.3 x 10^{-3}M) + SQ (1.0 x 10^{-4}M)</td>
<td>3186.7 ± 634.0</td>
<td>P &lt; 0.02</td>
</tr>
<tr>
<td>Serotonin (2.3 x 10^{-3}M) + Cy (2.8 x 10^{-9}M)</td>
<td>3007.5 ± 195.8</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>MSM (1.0 x 10^{-4}M)</td>
<td>5093.0 ± 352.6</td>
<td>NS</td>
</tr>
<tr>
<td>SQ (1.0 x 10^{-4}M)</td>
<td>2663.1 ± 536.9</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>Cy (2.8 x 10^{-9}M)</td>
<td>3315.7 ± 443.4</td>
<td>P &lt; 0.01</td>
</tr>
</tbody>
</table>

* Mean ± SEM of six experiments
** P values refer to the significance of different experiments with respect to the presence of 16.6 mM glucose
*** P values refer to the significance of different experiments with respect to the presence of 4.4 mM glucose

The results obtained with serotonin antagonists on the release of insulin differ from those of Feldman, Quickel Jr. and Lebovitz (1972). In contrast to the observations reported by these authors, we found that Cy did not potentiate the glucose-mediated insulin release and did not stimulate basal insulin release. Furthermore, we did observe a stimulation of basal insulin release with MSM using the same concentration as these authors. These discrepancies may be accounted for by differences in the experimental models used.

Considering the insulinotropic effects observed with MSM and SQ one may speculate that intracellular serotonin could be controlling the release of insulin by acting at the β cell membrane level rather than exerting a tonic inhibitory action on the migration...
of β cell granules, as postulated by Lebovitz and Feldman (1973). From our results it seems reasonable to hypothesize that serotonin which is stored in β cell granules (Jaim-Etcheverry and Zieher 1968) could be released together with insulin (Barath 1974) and by a feedback mechanism regulate the amount of insulin liberated at the β cell membrane. Serotonin antagonists could block this regulatory mechanism resulting in the observed increase in insulin release with both high and low glucose concentration. If this hypothesis is true, one may expect to find a decreased β cell sensitivity to serotonin in conditions characterized by basal insulin levels higher than normal and/or in those conditions with greatly augmented insulin release to insulino-tropic substances such as is the case with the hypersomatotrophic rat model (Åkerblom et al. 1972).

Our results with hypersomatotrophic rats show that their β cells have indeed a decreased sensitivity to serotonin. Exogenous serotonin inhibits the first phase of glucose-mediated insulin release from tumor-bearing rats but to achieve the same degree of inhibition as in normal rats, a serotonin concentration 20-fold higher had to be used.

The use of serotonin antagonists in hypersomatotrophic rats yielded different results than those in normal animals in that (1) MSM, SQ and Cy did not block the inhibition induced by serotonin on the glucose-mediated first phase of IRI release, (2) in the absence of exogenous serotonin, MSM did not potentiate the first phase of insulin release induced by glucose whereas SQ and Cy inhibited this first phase. These results can be interpreted in two ways, either changes have occurred in β cells of hypersomatotrophic rats leading to an alteration of their normal response to the serotonin antagonists or the concentration of the antagonists used were not sufficient to elicit a similar response as that obtained in normal rats. If this latter possibility proves to be correct it would appear that β cells in these animals have a decreased sensitivity to serotonin antagonists in addition to their decreased sensitivity to serotonin. The possibility that β cells from hypersomatotrophic rats might have a decreased sensitivity to serotonin antagonists is indicated by the fact that MSM and SQ although they stimulated the IRI release in the presence of a low glucose concentration, the levels of insulin obtained were 3 times lower than those seen in normal rats. The insulino-tropic effect observed with MSM and SQ in both normal and tumor-bearing rats is consistent with our earlier suggestion that these drugs appeared to act by antagonizing the action of intracellular serotonin released from β cells.

The present study does not exclude the possibility that in both normal and hypersomatotrophic rats the insulino-tropic effects of the serotonin antagonists might occur by an independent mechanism unrelated to serotonin.

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