INHIBITION OF INSULIN RELEASE BY CYPROHEPTADINE: EFFECTS ON 3',5'-CYCLIC-AMP-CONTENT AND 45Ca-ACCUMULATION OF INCUBATED MOUSE ISLETS

By

H. G. Joost, J. Beckmann, S. Lenzen and A. Hasselblatt

ABSTRACT

Cyproheptadine (1, 10 and 100 μM) significantly reduced insulin release from isolated mouse islets in response to glucose. In contrast, 1 mM cyproheptadine induced a large release of insulin into the incubation medium probably due to islet cell damage, since the islets had lost a considerable amount of their protein content. 3',5'-cyclic-AMP-levels of the islets were not significantly affected by 10 μM cyproheptadine in the presence as well as in the absence of theophylline (10 mM). As the inhibitory effect of cyproheptadine on insulin release was correlated with reduced accumulation of calcium-45, the agent may inhibit insulin release by interfering with the calcium handling of the β-cell.

Recent investigations revealed that cyproheptadine (Periactinol®, Nuran®) inhibits insulin release in vivo and in vitro (Joost et al. 1974; Feldman et al. 1974; Fischer & Rickert 1975). In the perfused rat pancreas the inhibition was partly overcome when the perfusion media contained theophylline, and when the calcium concentration of the perfusion media had been enhanced (Joost et al. 1976, in press).

The results have been presented in part at the 11th Annual Meeting of the European Association for the Study of Diabetes, Munich, September 1975.
Using isolated islets from obese mice we further investigated this inhibitory action in order to correlate cyclic-AMP content or the accumulation of calcium-45 with the effects of cyproheptadine.

Chemicals

Cyproheptadine-hydrochloride was obtained as a gift from Sharp and Dohme, München. 125I-labelled insulin was from Farbwerke Hoechst AG, Frankfurt, crude collagenase from Worthington, 45CaCl$_2$ was purchased from Amersham-Buchler, Braunschweig, bovine albumin (fraction V) and 2-[4-(2-Hydroxyäthyl)-piperaziny1-(1)]-äthansulfonsäure (HEPES) were obtained from Serva, Heidelberg, pure mouse insulin from Novo, Copenhagen. All other reagents (analytical grade) were from Merck AG, Darmstadt.

Incubation of the islets

Obese hyperglycaemic mice from our own colony (originated from a Bar Harbor strain and bred in our institute since 1966) were starved overnight, and anaesthetized by an intraperitoneal injection of pentobarbital (40 mg/kg). The pancreas was removed, cut into small pieces, and incubated for 15 min in 2 ml Krebs-Ringer-bicarbonate-buffer (KRB) containing 2 mg collagenase at 37°C with vigorous shaking. After centrifugation the islets were washed several times with buffer, dispersed in a Petri-dish and transferred into the incubation vials. In the experiments designed to investigate insulin release, four islets of approximately the same size were incubated per vial. When cyclic-AMP-levels were measured, batches of forty islets were transferred into each flask. The islets were pre-incubated in KRB with 3.3 mM glucose, 0.01% albumin, and 15 mM HEPES for 30 min, and afterwards for 15 or 60 min in the buffers containing 20 mM glucose and the various effectors.

Analytical methods

To measure the protein content the islets were washed twice in 0.9% NaCl at the end of the incubation period. The islets were dissolved in 1 N NaOH, and protein content was then measured by the slightly modified method of Lowry et al. (1951).

Immunoreactive insulin was assayed according to Zaharko & Beck (1968) using an antibody prepared in our laboratory, and pure mouse insulin as the reference. Samples for estimation of 3',5'-cyclic-AMP were prepared by the following procedure: At the end of the incubation period the buffer was removed and the vials were kept for 10 min in boiling water to destroy phosphodiesterase activity. After freeze drying 100 µl 1 N NaOH (containing 1% EDTA in order to dissolve earth alkaline ions) were added, and the samples were sonified and neutralized with 100 µl phosphoric acid. In control experiments we had assured that no breakdown of cyclic-AMP occurs under these conditions. The samples were then assayed in duplicate by the method of Brown et al. (1971) with the test combination of Amersham Buchler, Braunschweig.

Tracer experiments

The incorporation of calcium-45 was measured according to the method of Malaisse-Lagae & Malaisse (1971), and the results were corrected for the extracellular space (Hellman et al. 1971a,b). Batches of 5 islets were incubated in the presence of 20 mM

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glucose, calcium-45 (approximately 7.5 μCi/ml, resulting in a specific activity of approximately 3 μCi/μmol Ca), and [3H]sucrose (approximately 7 μCi/ml). After the incubation period of 15, 30 or 60 min the islets were washed 5 times in order to remove extracellular tracer, and dissolved in 200 μl NaOH (1% EDTA). The samples were neutralized by 1 N HCl and counted after addition of 5 ml Insta-Gel (Packard, Frankfurt). The count-rate was translated into pmol calcium by comparison with the specific activity of the buffers counted in parallel with each experiment, and corrected for the tracer in the sucrose space.

Calculations
The results are calculated as means ± SEM, and differences were tested for statistical significance by the U-test of Wilcoxon, Mann and Whitney.

RESULTS

Table 1 shows that 1,10, and 100 μM cyproheptadine significantly reduced insulin release from the incubated islets. In contrast, a large release of insulin into the medium was observed in the presence of 1 mM cyproheptadine. When the islets of these experiments were examined under the dissection microscope, they exhibited severe lesions. Furthermore, they had lost a considerable fraction of their protein content (Table 1).

The cyclic-AMP content of islets, when incubated with 10 μM cyproheptadine,

Table 1.
Effects of cyproheptadine on insulin release and protein content of incubated islets. Batches of 4 islets were incubated in the presence of 20 mM glucose. The results are means ± SEM of 10 observations.

<table>
<thead>
<tr>
<th>Cyproheptadine (mol/l)</th>
<th>IRI-release (ng/islet 60 min)</th>
<th>% of control</th>
<th>P</th>
<th>Protein content (μg/islet)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>none</td>
<td>30.8 ± 3.8</td>
<td>90</td>
<td>n. s.</td>
<td>2.7 ± 0.2</td>
<td>n. s.</td>
</tr>
<tr>
<td>10-7</td>
<td>27.3 ± 1.8</td>
<td></td>
<td></td>
<td>2.4 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>none</td>
<td>55.1 ± 8.1</td>
<td>45</td>
<td>&lt; 0.005</td>
<td>2.7 ± 0.3</td>
<td>n. s.</td>
</tr>
<tr>
<td>10-6</td>
<td>25.0 ± 7.7</td>
<td></td>
<td></td>
<td>2.5 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>none</td>
<td>51.3 ± 10.3</td>
<td>14</td>
<td>&lt; 0.001</td>
<td>3.4 ± 0.2</td>
<td>n. s.</td>
</tr>
<tr>
<td>10-5</td>
<td>7.1 ± 2.6</td>
<td></td>
<td></td>
<td>3.3 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>none</td>
<td>59.8 ± 7.5</td>
<td>21</td>
<td>&lt; 0.001</td>
<td>2.8 ± 0.2</td>
<td>n. s.</td>
</tr>
<tr>
<td>10-4</td>
<td>12.6 ± 1.1</td>
<td></td>
<td></td>
<td>3.3 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>none</td>
<td>62.5 ± 7.5</td>
<td>440</td>
<td>&lt; 0.001</td>
<td>3.8 ± 0.2</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>10-3</td>
<td>276.1 ± 10.9</td>
<td></td>
<td></td>
<td>2.2 ± 0.2</td>
<td></td>
</tr>
</tbody>
</table>

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Table 2.
Effects of cyproheptadine (10^{-5} M) and theophylline on insulin release and cyclic-AMP content of incubated islets. Batches of 40 islets were incubated for 15 or 60 min in the presence of 20 mM glucose. The results represent means ± sem of 6 experiments.

<table>
<thead>
<tr>
<th>Incubation time</th>
<th>Theophylline</th>
<th>IRI-release ng/islet</th>
<th>Cyclic-AMP fmol/islet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Cyproheptadine</td>
<td>Control</td>
</tr>
<tr>
<td>15 min</td>
<td>none</td>
<td>12.5 ± 2.3</td>
<td>8.14 ± 1*</td>
</tr>
<tr>
<td></td>
<td>10^{-2} M</td>
<td>24 ± 3.8</td>
<td>16.8 ± 2.5*</td>
</tr>
<tr>
<td>60 min</td>
<td>none</td>
<td>28.8 ± 6.5</td>
<td>10.4 ± 1.3*</td>
</tr>
<tr>
<td></td>
<td>10^{-2} M</td>
<td>57.5 ± 3.4</td>
<td>32.0 ± 5.7**</td>
</tr>
</tbody>
</table>

* P < 0.05.
** P < 0.01.

was not significantly altered (Table 2). 10 mM Theophylline enhanced insulin release, but failed to completely restore the secretory response: a significant inhibitory effect of cyproheptadine could still be observed.

The cyclic-AMP content of the islets was enhanced by theophylline. Although a slight decrease in the presence of cyproheptadine could be observed, again cyproheptadine failed to significantly affect the cyclic-AMP content.

To study the accumulation of calcium-45 by incubated islets we had combined the methods of Malaisse-Lagae & Malaisse (1971) and Hellman et al. (1971b), both washing the islets and correcting for the extracellular space. In the control experiments (Fig. 1) this procedure yielded data comparable to those of Hellman et al. (1971a).

As demonstrated in Fig. 1, 100, 10 and 1 μM cyproheptadine significantly decreased the incorporation of calcium-45 into incubated islets.

**DISCUSSION**

The results provide further evidence that cyproheptadine is a potent inhibitor of insulin release _in vitro_. 1 mM, in contrast, produced a large release of insulin. Similar findings have already been reported from incubation experiments with the rabbit pancreas (Feldman et al. 1972). Since we had never observed such large amounts of insulin being released from islets in response to any stimulus,
we suspected 1 mM cyproheptadine to destroy the islets. This assumption was supported by the observation that cyproheptadine caused visible lesions of the islets and loss of protein. Thus the insulin seems to be released into the medium by leakage rather than by an active secretion process. An obvious analogy of cyproheptadine with chlorpromazine and propranolol has to be outlined: the agents are known to inhibit insulin release, and produce large insulin release in higher concentration (Northrop et al. 1973; Ammon et al. 1973). As for chlorpromazine, islets cell damage accounts for this enhanced insulin release (Ammon et al. 1973).

The islet cyclic-AMP was not significantly affected by cyproheptadine. Theophylline increased insulin release and cyclic-AMP content of the islets in the absence as well as in the presence of cyproheptadine, although the inhibitory effect of cyproheptadine on insulin release was still visible. Thus sufficient ATP for the formation of cyclic-AMP is available. Furthermore, it can be concluded that cyproheptadine does not block the secretion which is produced by theophylline.

Hedeskov & Capito (1975) recently reported that caffeine can restore the reduced secretion of islets from starved mice. To account for this effect they offered two explanations: first, elevated cyclic-AMP, and secondly, effects of the phosphodiesterase-inhibitor on the calcium distribution of the cells. Brisson et al. (1972) have suggested that cyclic-AMP produces insulin release by translocating calcium from an organelle-bound pool to the cytoplasm. In view of this hypothesis cyproheptadine might inhibit the influx of calcium into the
β-cell, and theophylline could partly overcome the resulting lack of cytosolic calcium. This idea is supported by our finding that cyproheptadine inhibits the accumulation of calcium-45 into incubated islets.

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REFERENCES


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