Effects of disopyramide on insulin secretion in the perfusion of isolated rat pancreas in situ

Fumio Kimura and Setsuya Takeuchi
Department of Pharmacology, Nippon Medical School

Summary

The effects of disopyramide on insulin secretion were studied by perfusing the isolated rat pancreas in situ. In experiments carried out using Krebs-Ringer bicarbonate buffer containing 0.1% glucose (0.1% glucose buffer), the immunoreactive insulin (IRI) levels in the perfusate did not change after the administration of disopyramide (300 μg/0.1 ml, i.c.a.). When Krebs-Ringer bicarbonate buffer containing 0.3% glucose (0.3% glucose buffer) was substituted, IRI levels increased significantly after the disopyramide administration. The disopyramide-induced IRI rise was not affected by pretreatment with propranolol, phentolamine or atropine. The increase of insulin secretion induced by increasing glucose concentration from 0.1% to 0.3% in the perfusion fluid was suppressed by disopyramide. The suppressing action of disopyramide on glucose-stimulated insulin secretion was partially recovered after pretreatment with propranolol or phentolamine. Results show that disopyramide acts both as a stimulator and an inhibitor of insulin secretion.

Key words: disopyramide, insulin secretion, glucose-stimulated insulin secretion, propranolol, phentolamine

Introduction

Disopyramide (γ-di-isopropylamino-2-phenyl-2 (2-pyridyl)-butyramide phosphate, d.l. racemi), which is well known as an anti-arrhythmic, was previously reported to cause hypoglycemia. The mechanism inducing the hypoglycemia after the administration of disopyramide seems to be very complicated. In our previous report, we indicated that disopyramide has two action-mechanisms; one acts directly, stimulating the B-cells, while the other is an activation of the suppressing system for insulin secretion in the interacinar ganglia of the pancreas. It has been shown that the decrease of blood glucose levels is not related to plasma IRI after the administration of disopyramide in rats. Since the drug has no effect on blood glucose in depancreatized rats, the direct action of disopyramide on the pancreatic B-cells cannot be denied.

In the present study we have shown that disopyramide had a direct action on the pancreatic B-cells.

Materials and Methods

The experiments were conducted by perfusing isolated rat pancreas in situ, as described in...
our previous paper\textsuperscript{10}. Glucose was administered via either the perfusion fluid at a dose of 0.2 ml of 25% glucose solution or as a buffer solution including 0.3% glucose for 30 min. Disopyramide was injected into the perfusion fluid at a dose of 300\,\mu g/0.1 ml, 5 min before the glucose administration. IRI in the perfusate was determined by radioimmunoassay kit (Dainabot Lab.).

The following drugs were used: disopyramide phosphate (Nihon-Roussel Co.); propranolol hydrochloride (Sumitomo Chemical Co.); phentolamine mesylate (Ciba-Geigy (Japan) Co.); atropine sulphate (Iwaki Co.); hexamethonium bromide (C\textsubscript{6}, Yamanouchi Co.); and glucose (Wako Pure Chemical Co.). All compounds were dissolved in Krebs-Ringer bicarbonate solution.

**Results**

In the experiments using perfusion fluid containing 0.3% glucose, the IRI levels in the perfusate were significantly increased after administration of 300\,\mu g/0.1 ml of disopyramide (p<0.001). However, in the experiments using perfusion fluid containing only 0.1% glucose, the IRI levels did not change (Table 1a). Pretreatment with propranolol (10\,\mu g/ml), phentolamine (10\,\mu g/ml) or atropine (100\,\mu g/ml) did not significantly influence these rises in IRI levels induced by the administration of disopyramide in a solution of 0.3% glucose buffer (Table 1b).

When 0.1% glucose buffer was used, pretreatment with disopyramide significantly sup-

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**Table 1a** Effect of disopyramide on insulin secretion during the perfusion of Krebs-Ringer bicarbonate buffer containing 0.1 or 0.3% glucose (0.1 or 0.3% glucose buffer) in the isolated rat pancreas in situ

<table>
<thead>
<tr>
<th>Perfusion solution</th>
<th>No. of tests</th>
<th>IRI (\mu U/min) before</th>
<th>IRI (\mu U/min) after*</th>
<th>(\Delta)IRI</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1% glucose buffer</td>
<td>7</td>
<td>1.9± 1.9</td>
<td>0.1±0.14</td>
<td>-1.7±1.7</td>
</tr>
<tr>
<td>0.3% glucose buffer</td>
<td>32</td>
<td>100±15.8</td>
<td>127±18.6</td>
<td>27±5.5***</td>
</tr>
</tbody>
</table>

A dose of 300\,\mu g/0.1 ml of disopyramide was administered via the intra-ceolic artery. Each value represents the mean±S.E.

a) : maximal response. ***: p<0.001, significantly different according to the paired t-test.

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**Table 1b** Influences of pretreatment with certain blocking agents on the disopyramide-induced insulin hypersecretion with the perfusion of 0.3% glucose buffer in the isolated rat pancreas in situ

<table>
<thead>
<tr>
<th>Pretreatment blockers (\mu g/ml in perfusion fluid)</th>
<th>No. of tests</th>
<th>IRI (\mu U/min)</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Without blockade</td>
<td>With blockade</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>before</td>
<td>after*</td>
<td>difference (A)</td>
<td>before</td>
<td>after*</td>
</tr>
<tr>
<td>Propranolol (10)</td>
<td>6</td>
<td>122±28.3</td>
<td>185±32.9</td>
<td>63±8.8***</td>
<td>204±22.7</td>
<td>252±34.4</td>
</tr>
<tr>
<td>Phentolamine (10)</td>
<td>7</td>
<td>125±22.4</td>
<td>150±27.8</td>
<td>25±9.6*</td>
<td>243±39.3</td>
<td>286±52.0</td>
</tr>
<tr>
<td>Atropine (100)</td>
<td>7</td>
<td>10± 3.0</td>
<td>17± 3.3</td>
<td>7±1.4**</td>
<td>17± 4.9</td>
<td>24± 6.4</td>
</tr>
</tbody>
</table>

Each blocking agent was added to the perfusion fluid at a dose of 10~100\,\mu g/ml, starting 30 min before the administration of disopyramide. A dose of 300\,\mu g/0.1 ml of disopyramide was given via the intra-ceolic artery. Each value represents the mean±S.E.

a) : maximal response. b) : N.S., no significance using the Student's t-test. *p<0.05; **: p<0.005; ***: p<0.001, significantly different according to the paired t-test.
Fig. 1a Effect of disopyramide on insulin hypersecretion induced by additional glucose (25%, 0.2 ml) during the perfusion of 0.1% glucose buffer in the isolated rat pancreas in situ. Each point represents the mean ± S.E., and asterisks represent significant differences from the control values (*: p<0.05; **: p<0.01, Student's t-test). Number of tests is shown in parenthesis. i.c.a.: intra-celiac artery.

Fig. 1b Effect of disopyramide on insulin hypersecretion induced by additional glucose (25%, 0.2 ml) during the perfusion of 0.3% glucose buffer in the isolated rat pancreas in situ. Each point represents the mean ± S.E. Number of tests is shown in parenthesis. i.c.a.: intra-celiac artery.

pressed the rise in IRI levels that had invariably followed the administration of a 0.2 ml of 25% glucose solution (p<0.05–p<0.01, Fig. 1a). On the other hand, this suppressing action of disopyramide did not occur when the perfusion solution contained 0.3% glucose (Fig. 1b).

Fig. 2 represents the time course of the IRI levels after glucose stimulation. As seen in this Figure, the configuration of the curve shows as bi-phasic response. Disopyramide significantly suppressed both the first and second phase responses induced by the glucose stimulation (p<0.05–p<0.01). The suppressing action of disopyramide on glucose-stimulated insulin secretion significantly recovered after pretreatment with propranolol (first phase) or phentolamine (second phase) (p<0.05, Figs. 3 and 4). In contrast to this, pretreatment with hexamethonium did not cause any significant change in either the first or second phase-suppressing responses (Fig. 5).

Discussion

The initial hypoglycemia induced by disopyramide had previously been reported as possibly being insulin-independent. This hypothesis was derived from observations about the relation-
Fig. 2 Effect of disopyramide on insulin hypersecretion induced by perfusion of 0.3% glucose buffer in the isolated rat pancreas in situ.
Each point represents the mean ± S.E., and asterisks represent significant differences from the control values (*: p<0.05; **: p<0.01, Student's t-test). Number of tests is shown in parenthesis.
0.1% Gl. buf.: 0.1% glucose buffer. i.c.a.: intra-celiac artery.

Fig. 3 Influence of propranolol-pretreatment on disopyramide’s action on insulin hypersecretion induced by perfusion of 0.3% glucose buffer in the isolated rat pancreas in situ.
Propranolol was added to the pre-perfusion fluid at a dose of 10 μg/ml. Infusion was initiated 30 min before the administration of disopyramide. Each point represents the mean ± S.E., and the asterisk represents significant differences from the “with disopyramide” value (*: p<0.05, Student’s t-test). Number of tests is shown in parenthesis.
0.1% Gl. buf.: 0.1% glucose buffer. i.c.a.: intra-celiac artery.
Fig. 4 Influence of phentolamine-pretreatment on disopyramide's action on insulin hypersecretion induced by perfusion of 0.3% glucose buffer in the isolated rat pancreas in situ. Phentolamine was added to the pre-perfusion fluid at a dose of 10 μg/ml. Infusion was initiated 30 min before the administration of disopyramide. Each point represents the mean ± S.E., and asterisks represent significant differences from the "with disopyramide" values (*: p<0.05, Student's t-test). Number of tests is shown in parenthesis. 
0.1% Gl. buf.: 0.1% glucose buffer. i.c.a.: intra-celiac artery.

Fig. 5 Influence of hexamethonium (C6)-pretreatment on disopyramide's action on insulin hypersecretion induced by perfusion of 0.3% glucose buffer in the isolated rat pancreas in situ. C6 was added to the pre-perfusion fluid at a dose of 150 μg/ml. Infusion was initiated 30 min before the administration of disopyramide. Each point represents the mean ± S.E. Number of tests is shown in parenthesis. 
0.1% Gl. buf.: 0.1% glucose buffer. i.c.a.: intra-celiac artery.
ship between blood glucose and plasma IRI 2hs after the oral administration of disopyramide to rats. Results, however, obtained from experiments on pancreatectomized rats, suggested that the initial hypoglycemia may be pancreas-dependent. The present study, which indicates that disopyramide acts directly on the pancreas-islet, may solve the above discrepancy, since disopyramide has been observed to possess a mild accelerating action on insulin secretion from the islets. The action was found to be unaffected by pretreatment with certain blocking agents, such as phentolamine, propranolol or atropine. Consequently, disopyramide is considered to have acted directly on the B-cells in the islets. Clues to the mechanism of disopyramide's increasing action on insulin secretion may be found in the fact that no disopyramide action was detected in the experiments using 0.1% glucose buffer and that the increase in insulin secretion induced by additional glucose disappeared after administration of disopyramide. Fukazawa\textsuperscript{171} showed that when the pancreas-islets prepared by Lacy's method were exposed twice to a perifusion medium including 300µg/ml of disopyramide (the 2nd exposure was given 90 min after the 1st exposure), disopyramide did not produce any effect. Disopyramide seems to stimulate insulin secretion under certain conditions and to interfere with the response induced by the additional glucose.

On the other hand, disopyramide's depressing effect on glucose-stimulated insulin secretion recovered partially after pretreatment with propranolol or phentolamine. The effect of propranolol-pretreatment may be due to its action on the mechanism producing the sustained hypoglycemia\textsuperscript{169}. Phentolamine, on the other hand, noncompetitively antagonized disopyramide, since both the 1st and 2nd phase responses in glucose-stimulated insulin secretion recovered partially after pretreatment with phentolamine. It has been proposed that disopyramide may combine with the sympathetic α-receptor on the B-cell membrane, but the mode of interaction between disopyramide and phentolamine still remains unclear. Further experimentation on B-cell preparations is urgently needed.

The authors are grateful to Nihon-Roussel Co. for supplying Disopyramide phosphate.

**References**

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(Received for publication, April 17, 1987)