Adrenergic Modulation of Insulin and Glucagon Secretion from the Isolated Perfused Rat Pancreas

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Abstract

In order to observe the effect of the adrenergic system on pancreatic glucagon secretion in the isolated perfused rat pancreas, phenylephrine, an \(\alpha\)-adrenergic agonist, and isoproterenol, a \(\beta\)-adrenergic agonist, were added to the perfused solution. 1.2 \(\mu\)M phenylephrine suppressed glucagon secretion at 2.8 mM glucose, and it also decreased insulin secretion at 11.1 mM glucose. 240 nM isoproterenol enhanced glucagon secretion not only at 2.8 mM glucose, but also at 11.1 mM glucose, as well as insulin secretion at 11.1 mM.

In order to study the role of intra-islet noradrenalin, phentolamine, an \(\alpha\)-adrenergic antagonist, and propranolol, a \(\beta\)-adrenergic antagonist, were infused with the perfused solution. 10 and 100 \(\mu\)M phentolamine caused an increase in insulin secretion, and 25 \(\mu\)M propranolol decreased insulin secretion, while they did not cause any change in glucagon secretion.

From these results, it can be concluded that \(\alpha\)-stimulation suppresses not only insulin but also glucagon secretion, while \(\beta\)-stimulation stimulates glucagon secretion, as well as insulin secretion. Intra-islet catecholamine may have some effect on the B cell, whereas it seems to have no influence on the A cell.

There is general agreement concerning the adrenergic effect on the B cell of the pancreatic islet (Iversen, 1973; Woods and Porte, 1974; Gerich et al., 1974; Samol and Weir, 1979), but studies on adrenergic modulation of the A cell have not come to a common conclusion. For instance, it has been reported both that the influence of \(\beta\)-adrenergic agonism on A-cell secretion is stimulatory (Inversen, 1973; Gerich et al., 1974, Samols and Weir, 1979), and that it is inhibitory (Tyler and Kajinuma, 1972; Toyota et al., 1975). It has also been proposed that \(\alpha\)-adrenergic agonism inhibits glucagon secretion (Gerich et al., 1974), that it stimulates it (Toyota et al., Samols and Weir, 1979), and that it has no evident effect on it (Iversen, 1973).

Christensen and Iversen (1973) reported on the release of large amounts of noradrenalin from the isolated canine pancreas during glucose deprivation. As pancreatic islets receive an abundant nerve supply (Kern, 1971), the intra-islet release of noradrenalin is probably mainly due to an increase in noradrenalin secretion from the postganglionic nerve fibers.

The present study was undertaken with the isolated perfused rat pancreas to clarify the role of intra-islet catecholamine in the modulation of glucagon and insulin secretion, as well as to investigate the effect of \(\alpha\)-and \(\beta\)-adrenergic agonism on glucagon and insulin secretion.

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Materials and Methods

Male rats of the Wistar strain weighing between 200 and 300 g were used as pancreatic donors. The pancreatic perfusion was performed by the method of Toyota (1975) with minor modifications. The pancreas, along with the proximal portion of the duodenum, was isolated. A cannula placed in the celiac artery allowed the perfusate to go through the pancreas and the proximal portion of the duodenum. By means of another cannula inserted into the portal vein, all the perfusate was collected in tubes.

The perfusion medium was Krebs-Ringer bicarbonate buffer containing either 2.8 mM or 11.1 mM glucose, 4.5% dextran (Pharmacia Fine Chemical, Sweden) and 0.1% bovine serum albumin (Sigma Chemical Co., St. Louis, MO). The perfusate was kept from being recycled between the preparation of the pancreas and the solution reservoirs in order to avoid the gut hormones having an effect on insulin and glucagon secretion. The perfused solution was equilibrated with 95% oxygen and 5% CO₂, and it was stabilized at a temperature of 37°C. The flow rate of the perfusate was adjusted to 2.5 ml/min. All perfusate outflow from the portal vein was collected for 1 min. Immediately after sampling, each tube containing 2.5 ml of the perfusate, with 0.1 ml of Trasylol (1000 U) added, was placed in ice-water. They were frozen at -20°C until analyses.

The pancreas was perfused for an equilibration period of 10-20 min with a glucose concentration of either 2.8 or 11.1 mM, and then it was perfused with 1.2 μM phenylephrine hydrochloride (Cowa Co., Nagoya, Japan) or 240 nM isoproterenol (Nitsukin Chemical Co., Tokyo, Japan). They were added for 10-20 min at the glucose concentration of either 2.8 or 11.1 mM, respectively. 100 μM phentolamine (Ciba-Geigy Pharmaceutical Co., Switzerland) was infused for 10 min after infusion with 1.2 μM phenylephrine. 25 μM propranolol (Sumitomo Chemical Industry Co., Tokyo, Japan) was infused over a period of 10 min prior to the 240 nM isoproterenol infusion. In order to study the effect of intra-islet catecholamine on glucagon and insulin secretion, either 10-100 μM phentolamine or 25 μM propranolol was also added to the perfusate solution without adrenergic substances.

Analysis

The insulin concentration in the perfusate was measured by the double-antibody procedure using rat insulin as the standard (Hales et al., 1963). The glucagon concentration in the perfusate was measured by radioimmunoassay using antiserum 30 K of Unger (Luycky, 1972).

Results

The amount of insulin and glucagon secreted during 10 min in terms of the secretory amount per min are represented as $\Sigma IR_{I}$ and $\Sigma IR_{G}$, respectively.

1. Effect of phenylephrine during perfusion with glucose concentrations of 2.8 and 11.1 mM.

At a glucose concentration of 2.8 mM, $\Sigma IR_{I}$ was less than 1.5 ng/min and $\Sigma IR_{G}$ 385.8 ± 26.1 pg/min when 1.2 μM phenylephrine was added. When 100 μM phentolamine was then added to this, $\Sigma IR_{I}$ did not change, but $\Sigma IR_{G}$ was 496.5 ± 25.2 pg/ml. In comparison, $\Sigma IR_{I}$ was 1.4 ± 0.4 ng/min and $\Sigma IR_{G}$ 687.1 ± 6.1 pg/ml in the control. (Fig. 1, Table 1).

It can therefore be seen that at a glucose concentration of 2.8 mM, 1.2 μM phenylephrine did not have a clear effect on insulin secretion, because the insulin value is very low, being near the minimum measurable limit. However, it suppressed glucagon secretion, compared with that in the control ($P < 0.001$), and 100 μM phentolamine increased it ($P < 0.05$).

As for the perfusate solution containing 11.1 mM glucose, $\Sigma IR_{I}$ was 9.5 ± 1.8 ng/min and $\Sigma IR_{G}$ 173.9 ± 6.9 pg/min when 1.2 μM phenylephrine was infused. In the control, $\Sigma IR_{I}$ was 49.6 ± 1.4 ng/min, and $\Sigma IR_{G}$ 173.4 ± 6.0 pg/min. When 100 μM phentolamine was further added to the perfusate solution, $\Sigma IR_{I}$ was 51.9 ± 7.1 ng/min and $\Sigma IR_{G}$ 179.2 ± 6.7 pg/min (Fig. 2, Table 1).

Therefore, when the concentration of glucose in the perfusate was increased to 11.1 mM, 1.2 μM phenylephrine did not change glucagon secretion ($P > 0.1$), but
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Fig. 1. Effect of phenylephrine with phentolamine on insulin and glucagon release from the pancreases of rats at glucose 2.8 mM

Table 1. Effect of 1.2 μM phenylephrine on insulin and glucagon secretion with glucose concentrations of 2.8 and 11.1 mM

<table>
<thead>
<tr>
<th></th>
<th>glucose 2.8 mM</th>
<th>glucose 11.1 mM</th>
<th>glucose 2.8 mM</th>
<th>glucose 11.1 mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.2 μM phenylephrine</td>
<td>less than 1.5</td>
<td>9.5 ± 1.8 b)</td>
<td>385.8 ± 26.1 b)</td>
<td>173.9 ± 6.9</td>
</tr>
<tr>
<td>1.2 μM phenylephrine and phentolamine</td>
<td>less than 1.5</td>
<td>51.9 ± 7.1 c)</td>
<td>496.5 ± 25.2 b)</td>
<td>179.2 ± 6.7</td>
</tr>
<tr>
<td>Control</td>
<td>1.4 ± 0.4</td>
<td>49.6 ± 1.4</td>
<td>687.1 ± 6.1</td>
<td>173.4 ± 6.0</td>
</tr>
</tbody>
</table>

Values are means of 3–8 experiments ± SEM in this and the following table.
a) P < 0.001, effect of phenylephrine is significant.
b) P < 0.05, c) P < 0.001, effect on phentolamine is significant.
suppressed insulin secretion, as compared with that in the control (P<0.0001). 100 μM phentolamine caused the level of secretion to recover to the basal line (P<0.001).

2. Effect of isoproterenol during perfusion with glucose concentrations of 2.8 and 11.1 mM

At a glucose concentration of 2.8 mM, when the perfusate solution contained 240 nM isoproterenol, ΔIRI was 2.2±0.2 ng/min and ΔIRG 4427.5±289.7 pg/min, while in the control ΔIRI was 1.4±0.4 ng/min and ΔIRG 687.1±6.1 pg/min. When 25 μM propranolol was added prior to isoproterenol infusion, ΔIRI was 0.9±0.1 ng/ml and ΔIRG 747.9±41.4 pg/min (Fig. 3, 4, Table 2).

Therefore, when the perfusate solution contained 2.8 mM glucose, 240 nM isoproterenol did not change insulin secretion (P>0.1), but caused biphasic glucagon release (P<0.0001). However, when 25 μM propranolol was added prior to isoproterenol infusion, this biphasic glucagon release was not seen (P<0.001).

At a glucose concentration of 11.1 mM, when 240 nM isoproterenol was added, ΔIRI was 57.2±1.1 ng/min, and ΔIRG was 1154.9
Table 2. Effects of 240 nM isoproterenol on insulin and glucagon secretion with glucose concentrations of 2.8 and 11.1 mM

<table>
<thead>
<tr>
<th></th>
<th>Sum IRI (ng/min) glucose 2.8 mM</th>
<th>Sum IRG (pg/min) glucose 11.1 mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>240 nM isoproterenol</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.2 ± 0.2</td>
<td>57.2 ± 1.1 (c)</td>
</tr>
<tr>
<td></td>
<td>4427.5 ± 289.7 (d)</td>
<td>1154.9 ± 35.7 (e)</td>
</tr>
<tr>
<td>240 nM isoproterenol and 25 μM propranolol</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.9 ± 0.1</td>
<td>0.5 ± 0.1 (e)</td>
</tr>
<tr>
<td></td>
<td>747.9 ± 41.4 (d)</td>
<td>215.5 ± 33.2 (d)</td>
</tr>
<tr>
<td>Control</td>
<td>1.4 ± 0.4</td>
<td>49.6 ± 1.4</td>
</tr>
<tr>
<td></td>
<td>687.1 ± 6.1</td>
<td>173.4 ± 6.0</td>
</tr>
</tbody>
</table>

f), g) P<0.001, d) P<0.05, effect of isoproterenol is significant.
e), h, i) P<0.001, effect of propranolol is significant.
Glucose 2.8 mM and Propranolol 2.5 μM

Isoproterenol 240 nM

**Insulin**

\[
\text{IRI (ng/ml/min.)}
\]

\[
\begin{array}{c}
\text{N = 4} \\
\hat{\bar{\text{MEAN ± SEM}}}
\end{array}
\]

**Glucagon**

\[
\text{IRG (pg/ml/min.)}
\]

±35.7 pg/min, whereas in the control, ΣIRI was 49.6 ± 1.4 ng/min and IRG 173.4 ± 6.0 pg/min (Fig. 5, Table 2). However, when 25 μM propranolol was added to the perfusate solution beforehand, ΣIRI was 0.5 ± 0.1 ng/min and ΣIRG 215.5 ± 33.2 pg/min.

Therefore at a glucose concentration of 11.1 mM, 240 nM isoproterenol stimulated the release of insulin in a biphasic way (P < 0.05), and in spite of the high concentration of glucose, it stimulated glucagon secretion in a uniform way, overcoming its suppression by glucose (P < 0.001). The increase in insulin and glucagon secretion caused by isoproterenol was prevented (P < 0.001) by the addition of 25 μM propranolol prior to isoproterenol infusion.
3. Effect of phentolamine and propranolol alone during perfusion with glucose concentration of 2.8 and 11.1 mM.

At a glucose concentration of 2.8 mM, since the value of insulin was very low and near the minimum measurable limit, we could not make clear the effect of phentolamine and propranolol on insulin secretion. As for glucagon secretion, neither phentolamine nor propranolol had any influence (Table 3).

With a glucose concentration of 11.1 mM, when 100 µM phentolamine was added, ΣIRI was $104.9 \pm 5.0$ ng/min and ΣIRG $177.9 \pm 2.2$ pg/min (Fig. 6, Table 3), and with 10 µM phentolamine added, ΣIRI was $67.2 \pm 1.4$ ng/min and ΣIRG $202.4 \pm 16.6$ pg/min (Fig. 7, Table 3). In the control ΣIRI was $49.6 \pm 1.4$ ng/min and ΣIRG $173.4 \pm 6.0$ ng/min. Moreover, with the addition of 25 µM
Fig. 6. Effects of 100 μM phentolamine on insulin and glucagon release from the pancreases of rats at glucose 2.8 mM

Table 3. Effect of 10–100 μM phentolamine and 25 μM propranolol alone on insulin and glucagon secretion with glucose concentrations of 2.8 and 11.1 mM

<table>
<thead>
<tr>
<th></th>
<th>Σ IRI (ng/min)</th>
<th>Σ IRG (pg/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>glucose 2.8 mM</td>
<td>glucose 11.1 mM</td>
</tr>
<tr>
<td>10 μM phentolamine</td>
<td>1.6±0</td>
<td>67.2±1.4&lt;sup&gt;j&lt;/sup&gt;</td>
</tr>
<tr>
<td>100 μM phentolamine</td>
<td>less than 1.5</td>
<td>104.9±5.0&lt;sup&gt;k&lt;/sup&gt;</td>
</tr>
<tr>
<td>25 μM propranolol</td>
<td>0.3±0</td>
<td>10.2±0.7&lt;sup&gt;j&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control</td>
<td>1.4±0.4</td>
<td>49.6±1.4</td>
</tr>
</tbody>
</table>

<sup>j, k, l</sup> P<0.001, effect of antagonist is significant.
of propranolol, $\Sigma$IRI was $10.2 \pm 0.7$ ng/min and $\Sigma$IRG 186.2 ± 3.0 pg/min (Fig. 8, Table 3).

At a glucose concentration of 11.1 mM, neither phentolamine nor propranolol had any influence on glucagon secretion. However, 100 $\mu$M phentolamine ($P<0.001$), as well as 10 $\mu$M phentolamine ($P<0.001$) enhanced insulin release, and 25 $\mu$M propranolol suppressed insulin release ($P<0.001$).

Discussion

In the present experiments, $\alpha$-stimulation had a tendency to decrease glucagon secretion as well as insulin secretion. At a
glucose concentration of 2.8 mM, however, the suppressive effect of 1.2 μM phenylephrine on insulin secretion was not clear. This may be because the insulin secretion at that glucose concentration is very low and near the minimum measurable limit. At a glucose concentration of 11.1 mM the suppressive effect of 1.2 μM phenylephrine on glucagon secretion was vague. Here, the reason may be that the suppressive effect of glucose on glucagon secretion had almost reached the maximum at 11.1 mM, and the addition of phenylephrine could not have any more suppressive effect on it.

β-stimulation increased not only insulin secretion, but also glucagon secretion. At 11.1 mM glucose perfusion, the stimulative effect of 240 nM isoproterenol overcame the suppressive effect of glucose and caused the increase in glucagon secretion.
In the present study, $\alpha$-stimulation decreased not only insulin secretion, but also glucagon secretion. On the other hand, $\beta$-stimulation increased glucagon secretion as well as insulin secretion. As for the adrenergic effect on glucagon secretion, there is no general agreement. One reason may be that some experiment were done in vivo, while others were done in vitro, another reason may be that various kinds of species were used as experimental models, such as dogs, ducks, rats and humans.

Insulin secretion was decreased to 1/5 of that in the control with 1.2 $\mu$M phenylephrine, while it was increased to only 1.2 times that in the control with 240 nM isoproterenol. On the other hand, glucagon secretion was suppressed to only 1/2 of that in the control with 1.2 $\mu$M phenylephrine, whereas it was enhanced to 5 or 6 times that in the control with 240 nM isoproterenol.

Gerich et al. reported (1974) the possibility that the B cells may have predominantly $\alpha$-adrenergic receptors, whereas the A cells have a higher $\beta$-receptor population. The present data seems to agree with their hypothesis.

At 11.1 mM glucose, the administration of phentolamine alone showed enhanced insulin secretion, while the infusion of propranolol alone caused insulin secretion to be suppressed. As for glucagon secretion $\alpha$-and $\beta$-agonists did not show any effect on it at these concentrations.

According to Christensen and Iversen (1973), it is possible that the pancreas may contain an abundance of noradrenalin. With the addition of propranolol, the $\alpha$-stimulation of intra-islet noradrenalin may become predominant, and, as a result, insulin secretion may be suppressed. In contrast, with the addition of phentolamine, $\alpha$-stimulation may be weakened and insulin secretion may be enhanced. As our data showed the possibility that the B cells may have predominance in the $\alpha$-adrenergic receptor, the adrenergic modulation might usually work in inhibiting insulin secretion.

With regard to glucagon secretion, many studies have reported (Iversen, 1973; Toyota et al. 1975; Weir et al. 1974) that noradrenalin enhances glucagon secretion. Since autonomic nerve terminals are often anatomically adjacent to the A cells (Marliss et al., 1973), the reason why the administration of the blockers could not have worked is unclear. Even though the $\beta$-receptors of the A cells are predominant, increased insulin may have exerted an inhibitory effect on glucagon release during phentolamine infusion.

In our experiments, we used rather high concentrations of adrenergic agonists and antagonists. Because the pancreas has a rich nerve supply (Kern, 1971) and an abundance of noradrenalin (Christensen et al., 1973), these high concentrations might be physiologic. The concentration of isoproterenol we used is almost equivalent to that of the noradrenalin used by Weir et al. (1974). As for phenylephrine, Williams et al. (1976) reported that noradrenalin had 2.5 times the potency of phenylephrine in terms of $\alpha$-adrenergic response, so that 1.2 $\mu$M phenylephrine may have potency equivalent to 480 nM noradrenalin, which is near the concentration of 270 nM used by Weir et al., (1974).

References


