Alpha-2 adrenergic agonism stimulates islet glucagon release from perfused rat pancreas: possible involvement of alpha-2A adrenergic receptor subtype

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The sympathetic nervous system plays an important role in the regulation of pancreatic hormone release. It is generally accepted that insulin release is inhibited by alpha-2 adrenergic agonism (1–3) and stimulated by beta adrenergic agonism (4, 5) in both experimental animals and humans. In contrast, there is controversy with regard to adrenergic regulation of the pancreatic A cells. For example, it has been proposed that the effects of alpha-2 adrenergic agonism on glucagon release are stimulatory (5–7) or negligible (8, 9).

Alpha-2 adrenoceptors are subdivided into alpha-2A and alpha-2B subtypes using receptor binding methods (10–12) or cloning methodology (13, 14). The high affinities of oxymetazoline and WB-4101 for the alpha-2A subtype (12) have been demonstrated in studies on human platelets (13) and the cerebral cortex (11). Furthermore, the alpha-2 adrenoceptors in the human kidney (14) and neonatal rat lung (10) correspond to the alpha-2B subtype and have a high affinity for prazosin and chlorpromazine (12). Recent studies suggest that the inhibition of insulin release induced by alpha-2 adrenoceptor stimulation is mediated through the proposed alpha-2A subtype in isolated rat pancreatic islets (15, 16) and mice in vivo (17).

The present study was designed to confirm the involvement of the alpha-2 adrenergic receptor and its subtypes in glucagon release from the isolated, perfused rat pancreas.

Materials and methods

Experimental procedure

Pancreata of male Wistar rats weighing 300–400 g were isolated and perfused using the method of Grodsky and Fainska (18), as described previously (19, 20). Our institution’s guide for the care and use of laboratory animals was followed. The perfusate was Krebs-Ringer bicarbonate buffer supplemented with 4.5% (weight/volume) of dextran T-70 (Pharmacia LKB Biotechnology AB, Uppsala, Sweden), 1% (weight/volume) bovine serum albumin (Miles Inc., Kankakee, IL), 5.5 mmol/l glucose, 5 mmol/l sodium pyruvate, sodium fumarate and sodium glutamate (Sigma Co, St Louis, MO), and the flow rate was constant at 3.0 ml/min. The partial pressure of oxygen was maintained between 450 and 550 mmHg by a bubble oxygenator using a gas mixture of 95% O2 and 5% CO2. The perfusate pH was maintained between 7.35 and 7.45.
After a 15-min preperfusion period, insulin and glucagon responses to various concentrations of alpha-1 or alpha-2 agonists were examined. The effects of four alpha-2 antagonists on the alpha-2 agonism of clonidine were also examined. The sympathomimetic drugs and the blocking agents used were added to the perfusion buffer via an infusion pump (flow rate, 0.1 ml/min).

**Measurements of hormones**

Insulin was measured with a commercially available kit (Eiken Chemical Co Ltd, Tokyo, Japan) based on radioimmunoassay using rat insulin (Novo Research Institute, Bagsvaerd) as a standard. Glucagon was measured by a previously described method (21) using antiserum to synthetic glucagon 19–29 (22). The inter- and intra-assay coefficients of variance for insulin were 5.8 and 5.3%, respectively, and for glucagon 3.9 and 3.6%, respectively. Preliminary experiments showed that none of the chemicals at the final concentrations used interfered with the radioimmunoassays.

**Statistical analyses**

All values are expressed as means ± SEM, and the statistical significance of differences was evaluated using two-way analysis of variance (ANOVA) followed by Dunnett’s multiple comparison test (23). Differences were considered statistically significant when p < 0.05.

**Drugs**

The following drugs were used: methoxamine hydrochloride, phenylephrine hydrochloride, oxymetazoline hydrochloride and chlorpromazine hydrochloride (Sigma Co, St Louis, MO); clonidine hydrochloride (Tokyo Kasei Kogyo Co, Ltd, Tokyo, Japan); rauwolscine hydrochloride (Extrasynthese, Genay, France); WB-4101 hydrochloride (Research Biochemicals Inc., Natick, MA); and prazosin hydrochloride (Pfizer Pharm. Inc., Tokyo, Japan).

**Results**

**Effects of alpha adrenoceptor agonists on glucagon release**

As shown in Fig. 1 and Table 1, neither of the two alpha-1 selective agonists, methoxamine and phenylephrine, at concentrations of up to $10^{-6}$ mol/l affected glucagon release. In contrast, both the alpha-2A preferential agonist oxymetazoline and the non-subtype-selective partial alpha-2 agonist clonidine (10) induced concentration-dependent stimulation of glucagon release as compared with the basal levels, starting at $10^{-8}$ and...
Table 1. Effects of alpha adrenergic agonists on glucagon and insulin release from the perfused rat pancreas at 5.5 mmol/l glucose (means ± SEM, N=6; 8–10 min after initiation of the drug infusion).

<table>
<thead>
<tr>
<th>Alpha-agonist (mol/l)</th>
<th>0 (Basal)</th>
<th>10⁻⁹</th>
<th>10⁻⁸</th>
<th>10⁻⁷</th>
<th>10⁻⁶</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Glucagon (ng/min)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methoxamine</td>
<td>1.00±0.14</td>
<td>0.99±0.15</td>
<td>0.92±0.15</td>
<td>0.80±0.09</td>
<td>0.78±0.07</td>
</tr>
<tr>
<td>Phenylephrine</td>
<td>0.92±0.14</td>
<td>1.06±0.14</td>
<td>0.99±0.12</td>
<td>0.92±0.12</td>
<td>0.89±0.12</td>
</tr>
<tr>
<td>Oxymetazoline</td>
<td>0.90±0.09</td>
<td>1.21±0.12</td>
<td>2.85±0.34*</td>
<td>4.12±0.28*</td>
<td>4.32±0.25*</td>
</tr>
<tr>
<td>Clonidine</td>
<td>1.10±0.15</td>
<td>1.23±0.24</td>
<td>1.59±0.26</td>
<td>3.15±0.27*</td>
<td>3.39±0.44*</td>
</tr>
<tr>
<td><strong>Insulin (μU/min)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methoxamine</td>
<td>8.8±2.6</td>
<td>5.8±1.3</td>
<td>6.3±1.8</td>
<td>6.0±1.5</td>
<td>8.8±2.5</td>
</tr>
<tr>
<td>Phenylephrine</td>
<td>11.5±1.8</td>
<td>9.8±1.1</td>
<td>8.3±1.4</td>
<td>9.0±1.5</td>
<td>7.0±0.8</td>
</tr>
<tr>
<td>Oxymetazoline</td>
<td>12.8±3.8</td>
<td>9.0±1.0</td>
<td>7.0±1.6</td>
<td>7.5±2.1</td>
<td>8.8±2.3</td>
</tr>
<tr>
<td>Clonidine</td>
<td>6.3±1.2</td>
<td>6.5±1.8</td>
<td>8.0±2.0</td>
<td>6.8±1.8</td>
<td>10.8±2.0</td>
</tr>
</tbody>
</table>

* p<0.01 vs corresponding basal level.

10⁻⁷ mol/l, respectively (p<0.01). Although each of the four drugs at 10⁻⁷ or 10⁻⁶ mol/l induced an elevation in perfusion pressure, it was mild (less than 25% of the basal level) and the pressure stabilized within 5 min after each drug was administered.

**Effects of alpha-2 adrenergic antagonists on clonidine-induced glucagon release**

As shown previously, clonidine at 10⁻⁷ mol/l stimulated glucagon release to a level approximately threefold higher than the basal level. Therefore, we examined the effects of several alpha-2 antagonists on clonidine-induced glucagon release. Fig. 2 and Table 2 show that the non-subtype-selective alpha-2 antagonist rauwolscine at concentrations of 10⁻⁶ and 10⁻⁵ mol/l and the alpha-1 and alpha-2A selective antagonist WB-4101 (10, 12) at 10⁻⁵ mol/l significantly antagonized the effects of 10⁻⁷ mol/l clonidine as compared with corresponding controls. In contrast, neither the alpha-1 and alpha-2B selective antagonist prazosin nor the alpha-2B preferential antagonist chlorpromazine, at concentrations up to 10⁻⁵ mol/l antagonized the effects of clonidine.
Table 2. Antagonism of the effects of clonidine, by alpha-2 adrenoceptor antagonists, on glucagon and insulin-release from the perfused rat pancreas at 5.5 mmol/l glucose (means ± SEM, N = 6; 8–10 min after initiation of the drug infusion).

<table>
<thead>
<tr>
<th>Alpha-2 antagonist (mol/l)</th>
<th>Basal</th>
<th>Clonidine $10^{-7}$</th>
<th>$10^{-7}$</th>
<th>$10^{-6}$</th>
<th>$10^{-5}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucagon (ng/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None (Control)</td>
<td>0.93 ± 0.13</td>
<td>3.47 ± 0.32</td>
<td>3.26 ± 0.37</td>
<td>2.90 ± 0.29</td>
<td>2.41 ± 0.21</td>
</tr>
<tr>
<td>Rauwolscine</td>
<td>1.00 ± 0.06</td>
<td>4.31 ± 0.33</td>
<td>2.52 ± 0.18</td>
<td>1.20 ± 0.17&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.77 ± 0.13&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>WB-4101</td>
<td>0.88 ± 0.10</td>
<td>4.17 ± 0.58</td>
<td>4.49 ± 0.36</td>
<td>2.94 ± 0.27&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.91 ± 0.07&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Prazosin</td>
<td>1.30 ± 0.23</td>
<td>3.95 ± 0.40</td>
<td>3.61 ± 0.50</td>
<td>3.79 ± 0.44&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.32 ± 0.40</td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>1.30 ± 0.16</td>
<td>4.13 ± 0.74</td>
<td>3.80 ± 0.71</td>
<td>3.12 ± 0.57&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.99 ± 0.33</td>
</tr>
<tr>
<td>Insulin (µU/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None (Control)</td>
<td>9.0 ± 2.2</td>
<td>3.0 ± 1.0</td>
<td>3.8 ± 0.8</td>
<td>4.8 ± 1.1</td>
<td>6.0 ± 1.5</td>
</tr>
<tr>
<td>Rauwolscine</td>
<td>7.5 ± 1.6</td>
<td>4.8 ± 1.3</td>
<td>4.8 ± 1.3</td>
<td>7.8 ± 3.3</td>
<td>5.8 ± 1.8</td>
</tr>
<tr>
<td>WB-4101</td>
<td>3.3 ± 1.7</td>
<td>4.8 ± 2.1</td>
<td>6.5 ± 2.6</td>
<td>8.3 ± 2.3</td>
<td>8.5 ± 3.3</td>
</tr>
<tr>
<td>Prazosin</td>
<td>10.8 ± 3.4</td>
<td>5.0 ± 0.8</td>
<td>6.3 ± 2.0</td>
<td>8.0 ± 1.8</td>
<td>6.3 ± 1.9</td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>6.9 ± 3.1</td>
<td>5.1 ± 2.5</td>
<td>5.0 ± 2.3</td>
<td>5.2 ± 2.4</td>
<td>5.0 ± 2.3</td>
</tr>
</tbody>
</table>

<sup>a</sup>p < 0.05 and <sup>b</sup>p < 0.01 vs corresponding control.

Effects of alpha adrenoceptor agonists and alpha-2 adrenoceptor antagonists on insulin release

None of the eight drugs, at the concentrations tested, affected insulin release at a glucose concentration of 5.5 mmol/l (Tables 1 and 2).

Discussion

The results of the present study using alpha adrenoceptor agonists can be summarized as follows: The alpha-2A preferential agonist oxymetazoline induced a concentration-dependent stimulation of glucagon release more effectively than did the non-selective alpha-2 agonist clonidine, whereas the alpha-1 adrenoceptor agonists exerted no effects on glucagon release. We used oxymetazoline as an alpha-2 adrenergic agonist, because it has been reported (10) that this drug seems to be an antagonist in non-rodent tissues but an agonist in rodent tissues. In human platelets, oxymetazoline has an intrinsic activity of 0.09 (the intrinsic activity of a full agonist is 1.0 and that of an antagonist is 0) in inhibiting prostaglandin E<sub>1</sub>-stimulated adenylate cyclase activity (24), whereas it appears to be a full agonist in inhibiting intestinal secretions in the rat jejunum (25) and inhibiting insulin release from rat pancreatic islets (15). The reason for the discrepancy with Chan and Morgan’s observation (16), in which oxymetazoline was stated to be an alpha-2 antagonist in rat pancreatic islets, is not clear.

Because antagonists are better tools than agonists for studies of receptor subclassification (12), we also examined the effects of alpha-2 antagonists which have different selectivities for alpha-2A and alpha-2B subtypes. The non-selective alpha-2 antagonist and the alpha-2A preferential antagonist both antagonized clonidine-induced glucagon release, whereas the alpha-2B preferential antagonists failed to do so. Because basal insulin release was very low with 5.5 mmol/l glucose, we could not demonstrate any suppressive effects of alpha-2 agonists in these experiments. At a high glucose concentration (16.7 mmol/l), however, alpha-2 antagonists at $10^{-6}$–$10^{-7}$ mol/l markedly inhibited glucose-induced insulin release (data not shown).

Because our results were obtained from isolated pancreatic tissue, the possibility that secretory responses of A cells may be influenced by locally released hormones or by other intercellular interactions cannot be ruled out. Considering the recent suggestion (15–17) that the inhibition of insulin release induced by alpha-2 adrenoceptor stimulation is mediated through the alpha-2A subtype, it is possible that the effects of alpha-2 adrenergic agonism on glucagon release are indirect, via inhibition of insulin release. In the present study, however, glucagon release was apparently induced by alpha-2 agonists in the absence of changes in basal insulin release, thus suggesting that glucagon release was mediated through the direct stimulation of pancreatic A cells rather than through the inhibition of insulin release. In support of this, we present our recent finding (26) that alpha-2 adrenoceptor-induced inhibition of insulin release was mostly, though not completely, mediated through pertussis toxin-sensitive inhibitory guanine nucleotide-binding protein (Gi), whereas alpha-2 adrenoceptor-induced glucagon release did not seem to be mediated through Gi. Furthermore, we have shown (27) that the response of glucagon to $10^{-7}$ mol/l norepinephrine is present (ca. 55% of controls) even after the destruction of pancreatic B cells by streptozocin in rats. The discrepancy with previous reports (8, 9) concerning the effects of alpha-2 adrenergic agonism on pancreatic A cells seems to be attributable, in part, to species differences (8). However, the discrepancy between our data and Schuit’s (9), who used cultured purified A cells of rats, is not clear, but may be due to differences in experimental design.
Recently, the alpha-2 antagonists, midaglizole (DG-5128) (28, 29), SL 84.0418 (30) and MK-912 (31), as new oral anti-hyperglycemic agents, have been investigated in human in vivo studies. Midaglizole also inhibits epinephrine-induced human platelet aggregation in vitro (32) and in vivo (28). Considering that the human platelet is a representative of the alpha-2A adrenoceptor subtype, this agent seems to have at least the property of alpha-2A antagonist. Therefore, such agents may prevent both hyperglycemia and the development of diabetic complications associated with platelet aggregation.

In conclusion, we speculate that in rats islet glucagon release induced by alpha adrenoceptor agonism is mediated through alpha-2 adrenoceptors, possibly the alpha-2A subtype. These findings may further increase our understanding of the adrenergic regulation of islet hormones release and may provide a new therapeutic approach to diabetic patients.

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