Dopaminergic suppression of pancreatic somatostatin secretion

Mitsuyasu Itoh, Brian L. Furman and John E. Gerich

Endocrine Research Unit, Departments of Medicine and Physiology
Mayo Clinic and Mayo Medical School, Rochester, MN 55903, USA

Abstract. To characterize dopaminergic influences on pancreatic islet D cell function and its potential interaction with islet A and B cell function, the effect of dopamine (0.5–100 \( \mu \)m) on immunoreactive somatostatin (IRS), insulin (IRI), and glucagon (IRG) release from rat islets incubated in vitro was studied. Dopamine significantly suppressed the release of IRS (\( P < 0.001 \)) and IRI (\( P < 0.001 \)) and augmented IRG release (\( P < 0.001 \)). Maximum suppression of IRS and IRI release was evident at 20 \( \mu \)m dopamine with half-maximal suppression occurring at 0.5–1 \( \mu \)m. Maximal stimulation of IRG release was observed at 100 \( \mu \)m dopamine with a half-maximal response occurring at 5–10 \( \mu \)m. Suppression of IRS secretion by dopamine (20 \( \mu \)m) was completely reversed by the dopaminergic antagonists haloperidol (5 \( \mu \)m) and pimozide (5 \( \mu \)m), but was only partially reversed by the alpha adrenergic antagonist phentolamine (2 \( \mu \)m), and was further suppressed by the beta adrenergic antagonist propranolol (2 \( \mu \)m). Suppression of IRI release by dopamine was completely reversed by propranolol, but was unaffected by haloperidol, pimozide, and phentolamine. These results indicate that dopamine directly affects pancreatic islet D cell function, and that islet B and D cells appear to be more sensitive to dopamine than are A cells. Dopamine suppresses IRS secretion predominantly through activation of dopaminergic receptors, whereas it suppresses IRI release through an alpha adrenergic mechanism and stimulates IRG release through a beta adrenergic mechanism.

Histochemical studies indicate that endocrine cells of pancreatic islets contain dopamine (Cegrell 1968). The observations that this catecholamine inhibits insulin secretion (Feldman et al. 1971, 1972; Lebovitz & Feldman 1973; Quickel et al. 1971; Rossini & Buse 1973; Wong et al. 1967) and stimulates glucagon secretion (George & Rayfield 1974; Lorenzi et al. 1977, 1979; George & Bailey 1978) suggest that dopaminergic mechanisms participate in the regulation of islet A and B cell function. The effect of dopamine on pancreatic D cell function has not been extensively studied. In the only report to date (Barden et al. 1978), dopamine at a concentration that has alpha adrenergic effects inhibited somatostatin release from isolated rat islets, and this inhibition was reversed by (+)-butaclamol, a dopaminergic receptor antagonist. However, since the concentration of (+)-butaclamol used in that study (50 \( \mu \)m) also has alpha adrenergic antagonist activity (Caron et al. 1978; Robinson & Sulser 1976), and since alpha adrenergic receptor activation can suppress somatostatin release (Samols & Weir 1979; Sorenson et al. 1979), it is unclear whether dopamine suppresses somatostatin secretion by a dopaminergic or alpha adrenergic mechanism.

The present studies were undertaken to further characterize the effect of dopamine on secretion of pancreatic somatostatin and to determine its concomitant effects on islet A, B and D cell function. For this purpose, the dose-response relationship between dopamine and the release of somatostatin, insulin, and glucagon from isolated rat islets was investigated. In addition, since dopamine may have adrenergic as well as dopaminergic effects depending on the concentration of the amine used (Moran 1973), the effects of dopamine were studied in the presence of specific adrenergic and dopaminergic antagonists.

1 Address for principal author.
Materials and Methods

Adult male fed Wistar rats (280–340 g) were used in all experiments. Animals were anaesthetized by ip injection of sodium pentobarbital (60 mg/kg, Fort Dodge Laboratories, Fort Dodge, Iowa), and their pancreatic islets subsequently isolated by collagenase digestion according to the method of Lacy & Kostianovsky (1967). Groups of 20 islets were pre-incubated for 30 min at 37°C under a gas phase of 95% O2−5% CO2 in 0.2 ml of Krebs-Ringer bicarbonate buffer, pH 7.4, containing 3 mg/ml bovine plasma albumin (Armour Pharmaceutical Co., Phoenix, Arizona), 2 mM glucose (Sigma Chemical Co., St. Louis, Missouri), 5 mM fumarate, 5 mM pyruvate, 5 mM glutamate (Sigma), and trasyrol (1000 KIU/ml) aprotinin, Sigma). After removal of pre-incubation medium, islets were subsequently incubated in 1.2 ml of the same medium containing 8 mM glucose and test substances for 45 min as previously described (Gerich et al. 1979). Following incubation, aliquots of the medium were removed into pre-chilled tubes containing ethylenediamine tetra-acetic (EDTA) (4 mg/ml, Sigma) and were stored at −20°C until assay.

Drugs

Also added to the incubation media, as required, were: dopamine hydrochloride (0.5–20 μM, Intropin, Armour, Aquadilla, Puerto Rico); DL-norepinephrine·HCl (1 μM, Sigma); the dopaminergic antagonists haloperidol (5 μM, Haldol, McNeil Laboratories, Fort Washington, Pennsylvania) and pimozide (5μM, Sigma), the alpha adrenergic antagonist phenotolamine (2 μM, Regitine, Ciba Pharmaceutical Co., Summit, New Jersey), and the beta adrenergic antagonist propranolol (2 μM, Inderal, Ayerst Laboratories, New York, New York). Haloperidol was dissolved in lactic acid and pimozide was dissolved in tartaric acid. Final concentrations of lactic acid and tartaric acid were 1.88 × 10⁻³ and 8.65 × 10⁻³ per cent, respectively. Neither lactic acid nor tartaric acid at these concentrations affected the secretion of pancreatic hormones or hormone assays. Ascorbic acid (100 μM, Sigma) was also added in all experiments to prevent the degradation of dopamine. In preliminary studies it was determined that ascorbic acid itself did not influence the secretion of pancreatic hormone or hormone assays. All reagents were made up just prior to use and following their addition, incubation media was adjusted to pH 7.4.

Hormone assays

Insulin (IRI), glucagon (IRG), and somatostatin (IRI) were measured by radioimmunoassay as described previously (Gerich et al. 1979). Rat insulin, beef-pork glucagon (gifts of Dr. Mary Root, Eli Lilly Research Laboratories, Indianapolis, Indiana), and synthetic cyclic somatostatin (courtesy of Drs. Roger Guillemin and Jean Rivier, Salk Institute, San Diego, California) were used as standards.

Statistical analysis

Statistical analysis was performed using the non-paired Student’s t-test (two-tailed), and data in text and figures are expressed as mean ± SEM.

Results

Effect of dopamine on somatostatin, insulin and glucagon secretion: dose-response relationship (Fig. 1)

The effects of dopamine (0.5–20 μM) on IRS, IRI, and IRG secretion are given in Fig. 1. Dopamine
Effects of adrenergic and dopaminergic antagonists

Table 1.

Effect of adrenergic and dopaminergic antagonists on dopamine-induced changes in somatostatin (IRS), insulin (IRI), and glucagon (IRG) release from rat islets incubated in vitro (mean ± SEM).

<table>
<thead>
<tr>
<th></th>
<th>μM</th>
<th>N</th>
<th>IRS (pg/20 islets/45 min)</th>
<th>IRI (ng/20 islets/45 min)</th>
<th>IRG (pg/20 islets/45 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>16</td>
<td>182 ± 9</td>
<td>33.1 ± 2.5</td>
<td>1519 ± 93</td>
</tr>
<tr>
<td>Haloperidol</td>
<td>5</td>
<td>15</td>
<td>186 ± 10</td>
<td>34.3 ± 2.1</td>
<td>1631 ± 130</td>
</tr>
<tr>
<td>Propranolol</td>
<td>2</td>
<td>12</td>
<td>179 ± 8</td>
<td>34.5 ± 1.7</td>
<td>1578 ± 116</td>
</tr>
<tr>
<td>Phentolamine</td>
<td>2</td>
<td>14</td>
<td>189 ± 8</td>
<td>35.6 ± 1.6</td>
<td>1523 ± 143</td>
</tr>
<tr>
<td>Pimozide</td>
<td>5</td>
<td>13</td>
<td>187 ± 13</td>
<td>32.1 ± 4.5</td>
<td>1560 ± 129</td>
</tr>
<tr>
<td>Dopamine + Haloperidol</td>
<td>2</td>
<td>14</td>
<td>145 ± 10a</td>
<td>17.4 ± 2.2c</td>
<td>1865 ± 201</td>
</tr>
<tr>
<td>+ Propranolol</td>
<td>2</td>
<td>12</td>
<td>198 ± 7f</td>
<td>18.8 ± 3.0b</td>
<td>1962 ± 452</td>
</tr>
<tr>
<td>+ Phentolamine</td>
<td>2</td>
<td>11</td>
<td>192 ± 5f</td>
<td>29.4 ± 2.5e</td>
<td>1872 ± 293</td>
</tr>
<tr>
<td>Dopamine</td>
<td>20</td>
<td>28</td>
<td>120 ± 5c</td>
<td>13.5 ± 0.7c</td>
<td>2268 ± 122c</td>
</tr>
<tr>
<td>+ Haloperidol</td>
<td>5</td>
<td>11</td>
<td>177 ± 14i</td>
<td>13.9 ± 1.0c</td>
<td>2055 ± 160b</td>
</tr>
<tr>
<td>+ Propranolol</td>
<td>2</td>
<td>14</td>
<td>94 ± 9c,h</td>
<td>12.9 ± 1.3c</td>
<td>1692 ± 93b</td>
</tr>
<tr>
<td>+ Phentolamine</td>
<td>2</td>
<td>14</td>
<td>144 ± 11b,g</td>
<td>28.1 ± 1.9i</td>
<td>2174 ± 200b</td>
</tr>
<tr>
<td>+ Pimozide</td>
<td>5</td>
<td>11</td>
<td>176 ± 9i</td>
<td>13.6 ± 1.0c</td>
<td>2454 ± 172c</td>
</tr>
</tbody>
</table>

a = P < 0.05 vs control.  d = P < 0.05 vs 2 μM dopamine.  g = P < 0.05 vs 20 μM dopamine.
b = P < 0.01 vs control.  e = P < 0.05 vs 2 μM dopamine.  h = P < 0.01 vs 20 μM dopamine.
c = P < 0.001 vs control.  f = P < 0.001 vs 2 μM dopamine.  i = P < 0.001 vs 20 μM dopamine.

significantly suppressed the release of IRS (P < 0.001) and IRI (P < 0.001) and augmented IRG release (P < 0.001). Release of IRS and IRI was significantly suppressed by the lowest concentration of dopamine employed (0.5 μM), whereas IRG release was not significantly increased at dopamine concentrations less than 5 μM. Maximal suppression of IRS and IRI release was evident at 20 μM dopamine with half-maximal suppression occurring at 0.5—1 μM. Maximal effects on IRG release were observed at 100 μM dopamine (data not shown) with a half-maximal effect occurring at 5—10 μM.

Effects of adrenergic and dopaminergic antagonists

(Table 1)

Table 1 gives the effects of haloperidol, pimozide, propranolol, and phentolamine on changes in IRS, IRI, and IRG release observed in the presence of dopamine (2 and 20 μM). Neither the dopaminergic antagonists, haloperidol (5 μM) and pimozide (5 μM), nor the alpha and beta adrenergic antagonists, phentolamine (2 μM) and propranolol (2 μM), respectively, themselves altered hormone release. As was shown in other experiments (Fig. 1), dopamine significantly decreased IRS and IRI release at 2 and 20 μM while increasing IRG release at 20 μM. The suppression of IRS secretion by 2 and 20 μM dopamine was completely reversed by haloperidol and pimozide. Phenolamine completely reversed inhibition of IRS release by 2 μM dopamine and partially reversed that due to 20 μM dopamine. The suppression of IRI release by 2 and 20 μM dopamine was completely reversed by phentolamine and was unaffected by haloperidol and propranolol. The stimulation of IRG release by 20 μM dopamine was completely reversed by propranolol but was unaffected by haloperidol and phenolamine.

Effect of dopaminergic antagonists on norepinephrine-induced changes in somatostatin, insulin, and glucagon secretion

(Table 2)

To determine whether the reversal of dopamine suppression of IRS release by haloperidol and pimozide could have been due to alpha adrenergic antagonistic actions of these agents, the effects of haloperidol and pimozide on the suppression of IRS secretion induced by an alpha adrenergic agonist, norepinephrine (1 μM), were studied.
Norepinephrine reduced IRS and IRI release and stimulated IRG release. Neither haloperidol (5 μM) nor pimozide (5 μM) affected the norepinephrine-induced changes in IRS, IRI, and IRG release. However, phentolamine (2 μM) did reverse the effects of norepinephrine. These results indicate that at the concentrations used, haloperidol and pimozide do not have appreciable alpha adrenergic blocking effects.

Discussion

The present studies demonstrate that dopamine directly affects pancreatic islet D cell function: incubation of rat islets with this catecholamine resulted in suppression of somatostatin release along with concomitant inhibition of insulin release and stimulation of glucagon release. Half-maximum effects of dopamine on somatostatin and insulin release occurred at concentrations 10-fold lower than those required for half-maximal effects on glucagon secretion, suggesting that islet D and B cells are more sensitive to the effects of dopamine than islet A cells. Moreover, the present studies indicate that the effects of dopamine on islet A, B, and D cell function are mediated through different receptor mechanisms.

The suppressive effect of dopamine on somatostatin release was completely reversed by the dopaminergic antagonists haloperidol and pimozide, was partially reversed by phentolamine, and was augmented by propranolol when dopamine was studied at a concentration of 20 μM. Since phentol-amine, in addition to being an alpha adrenergic antagonist, is also a weak dopaminergic antagonist (William et al. 1976), these results are consistent with the conclusion that dopamine had acted predominantly through activation of dopaminergic receptors, although some of its inhibitory effects could be due to activation of alpha adrenergic receptors. The additional suppression of IRS release by propranolol is consistent with unmasking of some alpha adrenergic effects of dopamine. The suppressive effect of dopamine on insulin release was completely reversed by phentolamine but was unaffected by haloperidol, pimozide, and propranolol, and thus could be accounted for solely by alpha adrenergic mechanisms. The stimulatory effect of dopamine on glucagon release was completely reversed by propranolol and was unaffected by haloperidol, pimozide, and phentolamine; thus, the effect of dopamine on glucagon release could be accounted for solely by a beta adrenergic mechanism. Our observations that adrenergic mechanisms mediated the effects of dopamine on insulin and glucagon secretion are consistent with most (Lorenzi et al. 1977, 1979; George & Bailey 1978) but not all (George & Rayfield 1974) previous studies.

Dopaminergic suppression of islet somatostatin release observed in the present studies is consistent with the conclusion of Barden et al. (1978) who found that dopamine (100 μM) caused a 45 per cent reduction in somatostatin release from rat islets which was reversed by 50 μM (+)butaclamol. However, the results of that study are open to an alternative interpretation (i.e., dopamine may have

---

<table>
<thead>
<tr>
<th></th>
<th>μM</th>
<th>N</th>
<th>IRS (pg/20 islets/45 min)</th>
<th>IRI (ng/20 islets/45 min)</th>
<th>IRG (pg/20 islets/45 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>21</td>
<td>194 ± 10</td>
<td>33.6 ± 2.0</td>
<td>1564 ± 90</td>
</tr>
<tr>
<td>Haloperidol</td>
<td>5</td>
<td>16</td>
<td>201 ± 13</td>
<td>34.3 ± 3.3</td>
<td>1644 ± 147</td>
</tr>
<tr>
<td>Pimozide</td>
<td>5</td>
<td>9</td>
<td>190 ± 10</td>
<td>30.6 ± 4.2</td>
<td>1489 ± 182</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>1</td>
<td>22</td>
<td>130 ± 10b</td>
<td>16.6 ± 1.5b</td>
<td>2240 ± 915a</td>
</tr>
<tr>
<td>+ Haloperidol</td>
<td>5</td>
<td>12</td>
<td>133 ± 11b</td>
<td>17.6 ± 2.0b</td>
<td>2359 ± 196b</td>
</tr>
<tr>
<td>+ Pimozide</td>
<td>5</td>
<td>14</td>
<td>129 ± 15b</td>
<td>17.6 ± 1.5b</td>
<td>2518 ± 266b</td>
</tr>
<tr>
<td>+ Phentolamine</td>
<td>2</td>
<td>9</td>
<td>200 ± 8</td>
<td>30.4 ± 1.7</td>
<td>1367 ± 111</td>
</tr>
</tbody>
</table>

a = P < 0.01 vs control.  b = P < 0.001 vs control.
suppressed somatostatin by an alpha adrenergic mechanism) since 100 μM dopamine has potent alpha adrenergic effects (William et al. 1976; Ennis & Cox 1980) and since 50 μM (+)-butaclamol can act as an alpha adrenergic antagonist (Caron et al. 1978; Robinson & Sulser 1976). Although haloperidol has weak alpha adrenergic antagonist activity, pimozide at the concentrations used in the present study would be expected to be devoid of such actions (Ennis & Cox 1980). In other studies, dopamine has been reported to increase somatostatin release from rat hypothalamus in vivo (Chihara et al. 1979), rat cerebral cortex in vitro (Bennett et al. 1979), and cat gastric antrum in vitro (Uvnäs-Wallensten et al. 1978), whereas it has been found to decrease somatostatin release from rat hypothalamus in vitro (Bennett et al. 1979). The basis for this discrepancy between the effects of dopamine on somatostatin release in different tissues remains to be elucidated.

Previous studies employing epinephrine and norepinephrine have demonstrated that somatostatin release from the perfused dog pancreas is augmented by beta and is decreased by alpha adrenergic mechanisms. The results of the present studies are not inconsistent with these observations, although we could not unequivocally demonstrate alpha or beta adrenergic effects of dopamine on somatostatin release; the partial reversal of dopamine-induced inhibition by phenotamine in the present studies could have reflected cancellation of an inhibitory alpha adrenergic effect, unmasking of a stimulatory beta adrenergic effect or a combination of both processes. Preliminary evidence from our laboratory using epinephrine in the presence of specific adrenergic antagonists (Itoh et al. 1980a) indicates beta adrenergic stimulation augments while alpha adrenergic stimulation decreases somatostatin release from isolated rat islets.

Exogenous somatostatin is a potent inhibitor of insulin and glucagon secretion (Gerich et al. 1975). It has been suggested that endogenous somatostatin may regulate islet A and B cell function (Barden et al. 1977; Taniguchi et al. 1977; Itoh et al. 1980b). In the present studies, inhibition of somatostatin release by dopamine was accompanied by alpha adrenergic inhibition of insulin release and beta adrenergic stimulation of glucagon release. Reversal of the dopamine-induced inhibition of somatostatin release by haloperidol and pimozide did not further suppress insulin release nor did it decrease glucagon release. Thus, there was no evidence that changes in endogenous somatostatin release affected islet A and B cell function. Conceivably, however, under the present experimental conditions, the effects of dopamine could have predominated over those of endogenous somatostatin so that an interaction of islet D cells with islet A and B cell function was not evident.

Acknowledgments

The excellent technical help of T. Rambis, J. King, D. Stenner, and K. Greene is gratefully acknowledged. The work was supported in part by grants from the USPHS (AM2087), the Kroc Foundation, and the Mayo Foundation. Dr. Brian Furman was in receipt of a Wellcome Travel Grant.

References


Received on October 22nd, 1981.