Effects of galanin on the release of insulin, glucagon and somatostatin from the isolated, perfused dog pancreas

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Abstract. Galanin is a 29 amino acid peptide which has been found in intrapancreatic nerves. The effects of galanin, adrenergic and cholinergic blockade as well as somatostatin on the hormone release from the isolated perfused dog pancreas were studied. It was found that galanin dose-dependently inhibited insulin (P < 0.001) and somatostatin (P < 0.001) but not glucagon secretion at normal glucose levels. The lowest galanin concentration that caused a significant suppression of insulin and somatostatin secretion was 10^{-11} and 10^{-10} mol/l, respectively. Similar effects were evident during stimulation with 2.5 mmol/l arginine. Galanin (10^{-9} mol/l) caused a more pronounced inhibition of insulin and somatostatin secretion at high (10 mmol/l) and normal (5 mmol/l) than at low glucose (1.3 mmol/l). In contrast, suppression of the glucagon secretion was only seen at low glucose (1.3 mmol/l). Perfusion of 10^{-6} mol/l of atropine, phentolamine and propranolol had no effect on the galanin-mediated (10^{-10} mol/l) inhibition of insulin and somatostatin secretion. Galanin (10^{-12} – 10^{-10} mol/l) and somatostatin (10^{-12} – 10^{-10} mol/l) were equipotent in inhibiting insulin secretion whereas only somatostatin exerted a suppression of the glucagon secretion at normal glucose. Thus, galanin exerts a differential effect on islet hormone secretion and may participate in the hormonal control of insulin, glucagon and somatostatin secretion.

Galanin, a 29 amino acid peptide, was first isolated from porcine intestine (Tatemoto et al. 1983). This peptide, originally detected in nerves of the gut (Ekblad et al. 1985) and in the central nervous system (Rökaeus et al. 1984), has now been demonstrated in nerve fibres in association with the pancreatic islets (Dunning et al. 1986). Intravenous administration of galanin in conscious dogs produced hyperglycemia (Tatemoto et al. 1983; McDonald et al. 1985) and a concomitant decrease in plasma insulin (McDonald et al. 1985). The finding that galanin, when infused in vivo into the pancreatic artery, altered the insulin secretion points to a direct effect on the pancreatic B-cell (Dunning et al. 1986). Little work has so far been done on its pharmacological effect on glucagon (Dunning et al. 1986; McDonald et al. 1986; Lindskog & Ahrén 1987; Silvestre et al. 1987) and somatostatin secretion (Dunning et al. 1986; Silvestre et al. 1987).

The aims of the present study were: 1) to examine the direct effect of different doses of galanin on the release of glucagon, insulin and somatostatin from the isolated perfused dog pancreas; 2) to study the relationship between perfusate glucose levels and the glucagon, insulin and somatostatin responses to galanin; 3) to compare the effect of equimolar concentrations of galanin and somatostatin on the endocrine pancreas; and 4) to elucidate the mechanism of the action of galanin using adrenergic and cholinergic blockade.

Material and Methods

Preparation and perfusion media

Twenty-six mongrel dogs, fasted overnight, weighing...
16-31 kg, were used as pancreas donors. The technique for isolation of the pancreas and the perfusion system have previously been described in detail (Iversen & Miles 1971). In brief, the preparation consisted of the pancreas and the proximal 10 cm of the attached duodenum. A non-recirculating Krebs-Ringer bicarbonate buffer containing 40 g/l dextran (mol wt 75 000), 2 g/l bovine albumin, glutamate, fumarate and pyruvate, each at a concentration of 5 mmol/l, was pumped through the splenic and celiac arteries, and the total portal effluent was collected every min. The ionic composition of the standard perfusion medium was as follows (mmol/l): Na+, 140.0; K+, 4.4; Ca2+, 2.6; Mg2+, 1.8; Cl-, 124.9; HCO3-, 24.4; SO42-, 1.8 and H2PO4-, 1.1.

Oxygenation of the Krebs-Ringer bicarbonate buffer was achieved by means of a rotating roller screen in an atmosphere of 94.4% O2 and 5.6% CO2. During the experiments, the perfusion fluid had a constant pH of 7.4 and a temperature of 37°C. The perfusion pressure was 30-40 mmHg and the perfusion flow was 20 ml/min.

Experimental procedure

Samples were taken every min from the efflux. In order to prevent possible degradation of somatostatin and glucagon, 3 g/l EDTA were added to the tubes collecting the efflux. The samples were stored immediately at -18°C until assayed.

The pancreata (N = 26) were perfused for an equilibration period of 20-30 min. Thereafter the substances to be studied were infused for 10-30 min with 15 to 20 min recovery periods in between. The total perfusion averaged 3.5 h.

In the dose-response studies, four different concentrations of galanin (10^-12, 10^-11, 10^-10 and 10^-9 mol/l) were administered in random order to each of six pancreata in the absence of arginine and to each of four pancreata in the presence of 2.5 mmol/l arginine. In the study of the influence of the glucose level on galanin-mediated hormone responses, the glucose concentration (1.3, 5 and 10 mmol/l) was administered in random order to each of six pancreata. As for the study of the action of adrenergic and cholinergic blockers, the a-, ß-adrenergic and cholinergic blockers were given during a 30-min period in random order to each of four pancreata. Galanin was added for a 10-min period when the blocker had been infused for 10 min. To compare the potency of galanin and somatostatin on the pancreatic A- and B-cell secretion, three concentrations (10^-12, 10^-11 and 10^-10 mol/l) of each of the two peptides were infused in random order to each of six pancreata.

Reagents

Substances used in this study were galanin (Peninsula, Belmont, CA), somatostatin-14 (Peninsula, Belmont, CA), atropine sulphate (Sigma Chemical Co, St. Louis, MO), propranolol (ICI-Pharma, Cheshire, UK) and phentolamine (Ciba Pharmaceutical Co, Summit, NJ).

RIA

Somatostatin was measured by RIA as previously described (Hermansen et al. 1979; Hermansen 1980a) with tyrosine analogues of SR1H being iodinated with 125I. Insulin and glucagon were measured by specific and sensitive RIAs (Orskov et al. 1968). The reagents applied did not interfere in the RIAs.

Calculations

Owing to large interpancreatic differences in hormone output the relative (percentage) changes are also provided. Percentage change in hormone secretion (%): the mean of the 1-min hormone values during the galanin or somatostatin infusion (A) is related to the average of the last five 1-min values just before the addition of the peptides (B), i.e. \( \% = (A - B)/B \times 100\% \). Statistical analysis of the dose-response relationship was made using Page's test (Page 1963), which is a non-parametric paired test designed to test for the presence of trends in data. To test whether significant changes in hormone secretion occurred, Student's t-test for paired comparisons was used. In all cases 95% limits of confidence were applied to assess the significance of the difference.

Results

Effects of galanin at concentrations of 10^-12, 10^-11, 10^-10 and 10^-9 mol/l

The effects of galanin upon the release of glucagon, insulin and somatostatin were studied at a glucose concentration of 5.5 mmol/l in six perfusion experiments (Fig. 1). Galanin induced a dose-dependent inhibition of insulin (\( P < 0.001 \)) and somatostatin (\( P < 0.001 \)), whereas the glucagon secretion was unchanged. The lowest galanin concentrations that caused a significant suppression of insulin and somatostatin were 10^-11 mol/l (\( P < 0.001 \)) and 10^-10 mol/l (\( P < 0.01 \)), respectively. The galanin-induced inhibition of insulin and somatostatin was immediately and reversible.

Effects of galanin (10^-12 to 10^-9 mol/l) in the presence of arginine (2.5 mmol/l)

The effects of galanin (10^-12 to 10^-9 mol/l) on the A-, B- and D-cell secretion were also investigated in the presence of 2.5 mmol/l arginine and a glucose concentration of 5.5 mmol/l (Fig. 2). Again the neuropeptide elicited a dose-dependent sup-
Fig. 1.
Effects of galanin at concentrations of $10^{-12}$ (A), $10^{-11}$ (B), $10^{-10}$ (C) and $10^{-9}$ (D) upon the release of glucagon, insulin and somatostatin from the isolated, perfused dog pancreas. The glucose concentration was 5.5 mmol/l ($N = 6$, mean ± SEM).

Fig. 2.
Effects of galanin at concentrations of $10^{-12}$ (A), $10^{-11}$ (B), $10^{-10}$ (C) and $10^{-9}$ (D) upon the A, B, C and D cell secretion in the presence of 2.5 mmol/l arginine. The glucose level was 5.5 mmol/l ($N = 4$, mean ± SEM).
The effects of 10-min infusion of galanin (10^{-9} mol/l, D) on glucagon, insulin and somatostatin secretion from the isolated dog pancreas at low (1.3 mmol/l), normal (5 mmol/l) and high glucose (10 mmol/l) (N = 6, mean ± SEM).

pression of insulin (P < 0.001) and somatostatin secretion (P < 0.001) with thresholds at 10^{-11} mol/l (P < 0.01) and 10^{-10} mol/l (P < 0.01), respectively. Galanin did not cause any alteration in glucagon secretion in the presence of 2.5 mmol/l arginine.

**Effects of galanin at low, normal, and high glucose concentrations**

The effects of 10^{-9} mol/l galanin were investigated at low (1.3 mmol/l), normal (5 mmol/l), and high (10 mmol/l) glucose concentrations during six perfusion experiments (Fig. 3). At 1.3 mmol/l glucose, the insulin and somatostatin release was low and glucagon high, whereas the insulin and somatostatin release was augmented and glucagon secretion suppressed at the two highest glucose levels. At the low glucose of 1.3 mmol/l, galanin (10^{-9} mol/l) inhibited glucagon secretion by 48 ± 7% (P < 0.001), whereas no change in insulin and somatostatin secretion was discernable. On the other hand, galanin (10^{-9} mol/l) inhibited the insulin secretion by 71 ± 5% (P < 0.001) at normal and by 61 ± 6% (P < 0.001) at high glucose and the somatostatin release by 49 ± 7% (P < 0.001) at normal and by 36 ± 5% (P < 0.001) at high glucose, respectively. At the two highest glucose concentrations of 5 and 10 mmol/l, galanin did not cause any suppression of the glucagon secretion.

**Comparison of the effects of equimolar concentrations of galanin and somatostatin**

In each of six pancreas perfusions, the effect of 10-min infusions of equimolar galanin and somatostatin concentrations at 10^{-12}, 10^{-11} and 10^{-10} mol/l was compared in the presence of 5 mmol/l glucose and 2.5 mmol/l arginine. Fig. 4 demonstrates that galanin (10^{-12}, 10^{-11} and 10^{-10} mol/l) caused a dose-dependent inhibition of the insulin secretion (P < 0.01) by 16 ± 3, 22 ± 3 and 54 ± 8%, respectively. Somatostatin at equimolar concentrations likewise subdued the insulin secretion in a dose-dependent manner (P < 0.01) by 18 ± 3, 20 ± 3 and 55 ± 3%. Whereas galanin did not affect glucagon secretion, somatostatin eli-
Fig. 4.
Comparison of the effect of somatostatin at concentrations of $10^{-12}(A)$, $10^{-11}(B)$, $10^{-10}(C)$ (upper panel) and galanin at concentrations of $10^{-12}(A)$, $10^{-11}(B)$ and $10^{-10}(C)$ upon pancreatic glucagon and insulin release in the presence of 5 mmol/l glucose and 2.5 mmol/l arginine (N = 6, mean ± SEM).

Table 1.
Galanin-induced change (%) in pancreatic glucagon, insulin and somatostatin during α- and β-adrenergic or cholinergic antagonism (N = 4).

<table>
<thead>
<tr>
<th></th>
<th>Glucagon (%)</th>
<th>Insulin (%)</th>
<th>Somatostatin (%)</th>
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</thead>
<tbody>
<tr>
<td>Phentolamine ($10^{-6}$ mol/l)</td>
<td>$-8 \pm 6$ NS</td>
<td>$-51 \pm 2$ NS</td>
<td>$-25 \pm 7$ NS</td>
</tr>
<tr>
<td>Propranolol ($10^{-6}$ mol/l)</td>
<td>$17 \pm 10$ NS</td>
<td>$-58 \pm 4$ NS</td>
<td>$-22 \pm 4$ NS</td>
</tr>
<tr>
<td>Atropine ($10^{-6}$ mol/l)</td>
<td>$-14 \pm 9$ NS</td>
<td>$-54 \pm 8$ NS</td>
<td>$-32 \pm 10$ NS</td>
</tr>
<tr>
<td>Control</td>
<td>$-4 \pm 7$</td>
<td>$-66 \pm 8$</td>
<td>$-35 \pm 6$</td>
</tr>
</tbody>
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NS vs control.
cited a dose-dependent decrease in glucagon release ($P < 0.001$) by 8 ± 6, 33 ± 3 and 49 ± 8%, respectively. The glucagon threshold to somatostatin was $10^{-11}$ mol/l.

Effects of phentolamine, propranolol, and atropine on pancreatic glucagon, insulin and somatostatin release in response to galanin infusion

The effects of α-, β-adrenergic, and cholinergic blocking agents on galanin ($10^{-9}$ mol/l)-induced islet hormone secretion was investigated in four perfusion experiments. Thirty min infusion of either $10^{-6}$ mol/l phentolamine (α-adrenergic antagonist), $10^{-6}$ mol/l propranolol (β-adrenergic antagonist), or $10^{-6}$ mol/l atropine (cholinergic antagonist) did not significantly alter the amount of glucagon, insulin or somatostatin secreted at 5 mmol/l glucose.

As seen in Table 1, the α-, β-adrenergic, and cholinergic antagonism did not interfere with the galanin-mediated islet hormone secretion.

Discussion

Recent studies in dog (Dunning et al. 1986; McDonald et al. 1985, 1986; Manabe et al. 1986) and mouse (Lindskog & Åhrén 1987) revealed that infusion of galanin in vivo exerted a potent inhibition of the circulating insulin level and a concomitant elevation in blood glucose. The question arises whether the inhibitory effect of galanin on the insulin secretion is a direct one. Using the isolated, perfused dog pancreas we demonstrated that this seems to be so. We found that galanin ($10^{-12}$ to $10^{-9}$ mol/l) produced a dose-dependent inhibition of the release of insulin and somatostatin, whereas no change in glucagon was detected at a normal glucose level irrespective of arginine being present or not. It is not likely that the endocrine cells of the duodenal remnant of our preparation in any significant manner affect the islet hormone secretion in response to galanin, since identical release patterns of glucagon, insulin and somatostatin secretion have been obtained whether or not the duodenum was excluded during basal or stimulated conditions (Hermansen 1980b,c). The effect of galanin was modulated by the prevailing glucose level. Thus, a more pronounced inhibition of insulin and somatostatin secretion was observed at high and normal rather than at low glucose levels. Furthermore, a suppression of the glucagon secretion was seen at a low glucose level that would otherwise stimulate glucagon secretion. To which extent the differential sensitivity of pancreatic insulin, somatostatin and glucagon secretion to galanin is of physiological importance is still unknown. A rather big interpancreatic difference in the average levels of each hormone does explain some of the disparity in the hormone levels observed between the various series of experiments.

With respect to glucagon secretion, the reported effects of galanin diverse. The finding that the release of glucagon in vitro was unresponsive to the peptide in the presence of a normal glucose level corroborates previous results obtained in vivo in the dog (McDonald et al. 1985, 1986; Manabe et al. 1986) and in vitro in the perfused rat pancreas (Silvestre et al. 1987). Contrary to these observations, Dunning et al. (1986) and Lindskog & Åhrén (1987) found that the peptide caused a rise in circulating glucagon in the dog and mouse, respectively. The reason for this controversy is unknown. Since investigations in the dog gave contrary results, a species difference is not a very likely explanation.

Our demonstration in vitro of a galanin-induced suppression of the somatostatin secretion is in agreement with the findings in vivo (Dunning et al. 1986) and indicates that the peptide exerts a direct effect on both the B- and D-cell. The study shows furthermore that galanin on a molar basis is as efficient an inhibitor of the insulin secretion as is somatostatin. In accordance with our previous results, somatostatin equally potently inhibited glucagon and insulin secretion (Hermansen & Schwartz 1979), whereas galanin had no effect on glucagon secretion. In contrast to our results Silvestre et al. (1987) found that galanin in the perfused rat pancreas failed to alter the somatostatin release. The reason for this divergence is not known.

The mechanism behind the inhibitory action on insulin and somatostatin exerted by galanin remains to be established. To evaluate whether it could be due to an interaction with adrenergic and/or cholinergic receptors, we measured the ability of α-, β-adrenergic and cholinergic blockers to interfere with the effect of galanin. For this purpose, concentrations of blockers were chosen that in the isolated dog pancreas have previously been found effectively to counteract the action of...
α- and β-adrenergic as well as cholinergic agonists on pancreatic hormone release (Samols & Weir 1979; Iversen 1973). The finding that at these concentrations, adrenergic and cholinergic blockers did not significantly modify the effect of galanin on islet hormone release seems to rule out any major interaction with adrenergic and cholinergic receptors of this peptide. The mechanism of action of the galanin inhibition of islet hormone secretion could, however, be due to the occupancy of specific galanin receptors. In support of this Amiranoff et al. (1987) recently demonstrated highly specific galanin-binding sites at the insulin cell membrane indicating that a 54 000 mol wt protein is the pancreatic galanin receptor.

Galanin as well as vasoactive intestinal polypeptide, substance P, enkephalin, cholecystokinin, gastrin-releasing polypeptide, neuropeptide Y, and calcitonin gene-related peptide are contained in certain intrapancreatic nerves (Larsson 1979; Ahrén et al. 1986) and are all able to modulate islet hormone secretion (Ahrén et al. 1986; Hermansen 1980d; Hermansen 1983, 1984). The physiological role of the peptidergic nerves in general and the galaninergic nerves in particular in the regulation of the endocrine pancreas is difficult to assess because neither the mechanism of galanin release nor the amount released is known.

In conclusion, the physiological role of galanin as a hormone is obscure. It is conceivable that galanin reaches the islets by neural pathways and directly modifies pancreatic A-, B- and D-cell secretion. The observation that the glucagon secretion is only modified at a stimulated level may just reflect a differential sensitivity of the islet cells to galanin. Since galanin is present in the gut it may also be released to the blood stream in response to physiological stimuli and subsequently reach the pancreas.

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