Dual mode of action of glucose pentaacetates on hormonal secretion from the isolated perfused rat pancreas

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Leclercq-Meyer, Viviane, and Willy J. Malaisse. Dual mode of action of glucose pentaacetates on hormonal secretion from the isolated perfused rat pancreas. Am. J. Physiol. 275 (Endocrinol. Metab. 38): E610–E617, 1998.—Isolated perfused rat pancreases were exposed, in the presence of 10.0 mM L-leucine, to either α-D-glucose pentaacetate, β-L-glucose pentaacetate, or unesterified D-glucose, all tested at a 1.7 mM concentration. The pentaacetate ester of α-D-glucose, but not β-L-glucose, stimulated both insulin and somatostatin release, whereas unesterified D-glucose failed to do so. In the case of insulin output, the two esters differed from one another not solely by the magnitude of the secretory response but also by its time course and reversibility. Compared with these data, the most salient difference found in the case of somatostatin release consisted of the absence of an early secretory peak in response to α-D-glucose pentaacetate administration and the higher paired ratio between the secretory responses evoked by the esters of glucose and by unesterified D-glucose (5.5 mM) administered at the end of the experiments. The two esters provoked an initial and short-lived stimulation of glucagon secretion, in sharp contrast to the immediate inhibitory action of unesterified D-glucose. Thereafter, α-D-glucose pentaacetate, but not β-L-glucose pentaacetate, caused inhibition of glucagon release, such an effect being reversed when the administration of the ester was halted. These findings indicate a dual mode of action of glucose pentaacetate esters on hormonal secretion from the endocrine pancreas. The intracellular hydrolysis of α-D-glucose pentaacetate and subsequent catabolism of its hexose moiety may contribute to the early peak-shaped insulin response to this ester, to the persistence of a positive secretory effect in B and D cells after cessation of its administration, and to the late inhibition of glucagon release. However, a direct effect of the esters themselves, by some as-yet unidentified coupling process, is postulated to account for the stimulation of insulin and somatostatin release by β-L-glucose pentaacetate and for the initial enhancement of glucagon secretion provoked by both glucose esters.

Insulin secretion; somatostatin secretion; glucagon secretion

Several esters of monosaccharides were recently introduced as tools to increase the nutritional value and/or biological efficiency of the corresponding carbohydrates. This approach is inspired by the consideration that the esters cross the plasma membrane without requiring the intervention of a specific transport system and then undergo intracellular hydrolysis, so that the sugar moiety becomes readily available to act as a nutrient or metabolic inhibitor (13). For instance, α-D-glucose pentaacetate is better metabolized and displays a higher insulinotropic action than unesterified D-glucose (18, 20). Likewise, D-mannohexitolose hexaacetate inhibits D-glucose metabolism in cells otherwise resistant to the heptose (5, 15). The tetraacetate ester of 2-deoxy-D-glucose is also more potent than unesterified 2-deoxy-D-glucose as either an inhibitor of D-glucose metabolism and insulinotropic action in pancreatic islets (21) or as a cytostatic agent in several lines of tumoral cells (2, 10, 11, 19).

Surprisingly, however, a few esters of hexoses that are either not metabolized (e.g., β-L-glucose pentaacetate) or even inhibitors of D-glucose metabolism (e.g., 2-deoxy-D-glucose tetraacetate) were also found to stimulate insulin release under suitable experimental conditions (12, 14, 18). Several findings indicate that such a situation cannot be accounted for by the metabolism of the acetate moiety of these esters. For example, β-D-galactose pentaacetate, which is as efficiently taken up and hydrolyzed in pancreatic islets as α-D-glucose pentaacetate (20, 22), fails to display any insulinotropic action. Moreover, the secretory response to the latter ester is not duplicated by the combination of unesterified D-glucose and either acetic acid or its methyl or ethyl esters (13, 18). The unexpected observation that esters of hexoses devoid of nutritional value may stimulate insulin release has led to the proposal that such esters might themselves act directly on pancreatic islet B cells at the intervention of specific receptors in a manner somehow comparable to that involved in the recognition of bitter compounds by taste buds (17).

In light of these considerations, the major aim of the present study was to search for further evidence in support of the postulated dual mode of action of selected hexose pentaacetates on insulin release from the endocrine pancreas. For such a purpose, the effects of α-D-glucose pentaacetate and β-L-glucose pentaacetate on the secretion of insulin, glucagon, and somatostatin were compared with those of unesterified D-glucose in isolated perfused rat pancreases.

MATERIALS AND METHODS

The pentaacetate esters of α-D-glucose and β-L-glucose were purchased from Sigma Chemical (St. Louis, MO).

Fed female Wistar rats (B & K Universal, Hull, UK) were anesthetized with pentobarbital sodium, and the pancreas was perfused free from adjacent organs through both the celiac and superior mesenteric arteries, as described elsewhere (16).

The basal salt-balanced perfusion medium contained L-leucine (10.0 mM), dextran (clinical grade; 40 g/l), and BSA (RIA grade; 5 g/l). It also contained, as required, α-D-glucose pentaacetate (1.7 mM), β-L-glucose pentaacetate (1.7 mM), or unesterified D-glucose (1.7 or 5.5 mM).
The techniques used for the measurement of plasma glucose and insulin concentrations; perfusion pressure; and pancreatic insulin, glucagon, and somatostatin content and release were identical to those mentioned elsewhere (7, 8). The concentration of o-glucose in the effluent was measured by the hexokinase/glucose-6-phosphate dehydrogenase method (1).

The oscillatory pattern of hormonal release was assessed by a procedure previously reported (6). In each individual experiment, the mean hormonal output over a given period of time was calculated from all measurements made over that period. The basal hormonal output was computed from min 20 to min 25, inclusive. The absolute values for such a basal output were, as a rule, not significantly different from one another in the three series of experiments conducted with Pentaacetate, Pentaacetate, or glucagon. The basal output of glucagon was somewhat higher (P < 0.05), however, in the experiments with α-D-glucose pentaacetate than with β-L-glucose pentaacetate. Table 1 lists the characteristics of rats and experimental variables.

All results are presented as means ± SE together with the number of individual observations (n). The significance of differences between mean values was assessed by use of Student’s t-test and ANOVA.

RESULTS

In the experiments conducted with unesterified D-glucose, the concentration of the hexose in the effluent progressively increased from 0.27 ± 0.03 mM at min 28 to 1.69 ± 0.05 mM at min 34 and decreased from 1.71 ± 0.07 mM at min 53 to 0.34 ± 0.01 mM at min 58. These measurements allowed for correction for the dead space of the perfusion device. Hence, as seen in Figs. 1–6, the vertical dotted lines correspond to the time at which a change in D-glucose concentration was first detected in the effluent medium collected from the perfused pancreas.

In the experiments conducted in the presence of either α-D-glucose pentaacetate or β-L-glucose pentaacetate, no sizable increase (>0.02 mM) in the α-glucose concentration of the effluent was observed in response to the administration of the ester.

The perfusion pressure was not affected by the administration of either D-glucose or its esters (data not shown).

The administration of α-D-glucose pentaacetate (1.7 mM) provoked a biphasic increase in insulin output, with slow oscillations during the late period of exposure to the ester (Fig. 1, top). For instance, after the initial peak at min 34–35, a second secretory peak was recorded at min 42–43, i.e., 7.5 ± 0.3 min later. After the first peak, the hormonal output reached, within 3.5 ± 0.3 min, a nadir value, which corresponded to a 66.9 ± 5.0% fractional decrease in secretory rate, whereas the subsequent rise in insulin release after this first nadir corresponded to a 160.9 ± 19.5% relative increment in secretory rate (n = 4 in all cases). A third reascension in insulin output was observed after the second nadir (min 45.0 ± 0.4), the hormonal output again increasing over 4 min by 60.3 ± 15.5% above the paired preceding value. As computed over a period of 22 min (min 29–50), the integrated amount of insulin released during stimulation by the ester averaged 98.8 ± 14.2% (n = 4) of the paired value recorded at the end of the experiments (min 79–100) in response to the administration of 5.5 mM unesterified D-glucose.

The administration of β-L-glucose pentaacetate (1.7 mM) also resulted in an increase in insulin output. However, the mean output of insulin during exposure to β-L-glucose (min 29–53, inclusive) averaged no more than 0.43 ± 0.05 ng/min (n = 4), as distinct (P < 0.01) from 6.98 ± 1.54 ng/min (n = 4) in the case of α-D-glucose pentaacetate. Likewise, between min 46 and 50, the output of insulin, when expressed relative to the paired basal value (min 20–25), averaged no more than 6.4 ± 1.9 (n = 4) during exposure to β-L-glucose pentaacetate, as compared (P < 0.05) with 63.2 ± 21.9 (n = 4) in the case of α-D-glucose pentaacetate (Fig. 2).

The secretory responses of the B cells to each of these two esters also differed from one another by their time courses. Thus, whereas an early secretory peak reaching its zenith value at min 34–35 was always recorded during stimulation by α-D-glucose pentaacetate, such apparently was not the case in the pancreases exposed to β-L-glucose pentaacetate. Nevertheless, in the latter experiments, the secretory rates recorded 3 min before and 3 min after the highest value found at min 33–35 averaged, respectively, 46.8 ± 15.2 and 75.8 ± 6.6% of such a value, suggesting the possible occurrence of a modest early phasic response.

The experiments conducted in the presence of α-D-glucose pentaacetate and β-L-glucose pentaacetate also differed from one another by the pattern of reversibility when the administration of the esters was interrupted at min 53. After exposure to β-L-glucose pentaacetate,

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**Table 1. Characteristics of rats and experimental variables of perfusions conducted with α-D-glucose pentaacetate, β-L-glucose pentaacetate, or D-glucose**

<table>
<thead>
<tr>
<th>Characteristics/Variables</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat weight, g</td>
<td>202 ± 3</td>
</tr>
<tr>
<td>Plasma</td>
<td></td>
</tr>
<tr>
<td>Glucose, mM</td>
<td>4.6 ± 0.1</td>
</tr>
<tr>
<td>Insulin, µU/ml</td>
<td>502 ± 4.5</td>
</tr>
<tr>
<td>Pancreas</td>
<td></td>
</tr>
<tr>
<td>Wet weight, g</td>
<td>0.805 ± 0.036</td>
</tr>
<tr>
<td>Insulin content</td>
<td></td>
</tr>
<tr>
<td>µg</td>
<td>90.5 ± 6.0</td>
</tr>
<tr>
<td>µg/µl</td>
<td>112.1 ± 4.8</td>
</tr>
<tr>
<td>Glucagon content</td>
<td></td>
</tr>
<tr>
<td>µg</td>
<td>5.1 ± 0.5</td>
</tr>
<tr>
<td>µg/µl</td>
<td>6.3 ± 0.5</td>
</tr>
<tr>
<td>Somatostatin content</td>
<td></td>
</tr>
<tr>
<td>µg</td>
<td>0.37 ± 0.04</td>
</tr>
<tr>
<td>µg/µl</td>
<td>0.46 ± 0.04</td>
</tr>
<tr>
<td>Molar ratio</td>
<td></td>
</tr>
<tr>
<td>Insulin/glucagon</td>
<td>10.9 ± 0.9</td>
</tr>
<tr>
<td>Insulin/somatostatin</td>
<td>73.4 ± 7.5</td>
</tr>
<tr>
<td>Glucagon/somatostatin</td>
<td>6.9 ± 0.7</td>
</tr>
<tr>
<td>Flow rate, ml/min</td>
<td>1.44 ± 0.02</td>
</tr>
<tr>
<td>Perfusion pressure, mmHg</td>
<td></td>
</tr>
<tr>
<td>Min 20</td>
<td>26.2 ± 1.0</td>
</tr>
<tr>
<td>Min 100</td>
<td>28.9 ± 1.2</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 12.
the output of insulin, whether expressed in absolute terms or relative to basal value, rapidly declined to a level close to that found after administration of unesterified D-glucose. Indeed, in the presence of the unesterified hexose, the output of insulin, when expressed relative to paired basal value, averaged only 1.3 ± 0.5 (n = 4) between min 46 and 50.

The functional integrity of the perfused pancreases was assessed at the end of the experiments by monitoring the secretory response to 5.5 mM unesterified D-glucose. In all cases, the hexose evoked a biphasic increase in insulin release, with typical oscillations during the late phase of stimulation. The functional response to the sugar appeared somewhat higher after prior stimulation with either α-D-glucose pentaacetate or β-L-glucose pentaacetate than after a prior administration of unesterified D-glucose (Fig. 1). Such differences failed, however, to achieve statistical significance, the mean value for insulin release between min 70 and 100 averaging 6.61 ± 1.65, 4.46 ± 0.62, and 3.52 ± 0.90 ng/min after prior exposure to α-D-glucose pentaacetate, β-L-glucose pentaacetate, and unesterified D-glucose, respectively.

The secretory data concerning the release of somatostatin were, in three respects, comparable to those
obtained in the case of insulin output. Thus α-D-glucose pentaacetate and, to a lesser extent (P < 0.005), β-L-glucose pentaacetate, but not unesterified D-glucose, augmented somatostatin output (Fig. 3). Such a difference remained obvious when the release of somatostatin was expressed relative to its paired basal value. For instance, the secretory peak recorded at min 81 averaged, relative to paired basal output, 2.99 ± 0.49 and 2.02 ± 0.87 after prior administration of α-D-glucose pentaacetate and β-L-glucose pentaacetate, respectively, as distinct (P < 0.05) from only 0.83 ± 0.26 after prior exposure of the pancreas to unesterified D-glucose.

The secretory response to the esters largely exceeded, however, that evoked at the end of the experiments by 5.5 mM D-glucose, at variance with the situation found in the case of insulin output. Another marked difference between insulin and somatostatin output consisted of the virtual absence of an early somatostatin secretory peak in the pancreases exposed to α-D-glucose pentaacetate. The absence of an early stimulation of somatostatin release by the glucose esters contrasted with the occurrence of an early secretory peak when the pancreas was exposed to unesterified D-glucose at the end of the experiments. Indeed, in the latter situation, an initial peak-shaped response was always observed, even after prior exposure to unesterified D-glucose. Thus the output of somatostatin

Fig. 3. Time course for changes in somatostatin output. Same presentation as in Fig. 1.

obtained in the case of insulin output. Thus α-D-glucose pentaacetate and, to a lesser extent (P < 0.005), β-L-glucose pentaacetate, but not unesterified D-glucose, augmented somatostatin output (Fig. 3). Second, when the administration of the esters was interrupted at min 53, the output of somatostatin rapidly declined, reaching between min 58 and 72 a mean level somewhat higher, albeit not significantly so, after exposure to α-D-glucose pentaacetate (4.6 ± 0.9 pg/min) or β-L-glucose pentaacetate (6.8 ± 3.2 pg/min) than after administration of unesterified D-glucose (1.7 ± 0.3 pg/min). Expressed relative to paired basal value, such secretory rates averaged, respectively, 1.31 ± 0.19 as distinct (P < 0.001) from 0.55 ± 0.12 and 0.36 ± 0.09

Fig. 4. Time course for changes in somatostatin output evoked by α-D-glucose PA ( ), β-L-glucose PA ( ), and unesterified D-glucose ( ), hormonal output being expressed as percentage of paired basal value (min 20–25). Values (means ± SE) refer in each case to same 4 individual experiments as those illustrated in Fig. 3.
at min 79 and 83, respectively, averaged, relative to the paired value recorded at min 81, 15.5 ± 8.7 and 12.9 ± 14.2% after prior stimulation by α-D-glucose pentaacetate, 32.4 ± 6.1 and 49.2 ± 11.7% after prior delivery of β-L-glucose pentaacetate, and 36.8 ± 5.1 and 34.2 ± 9.1% after prior administration of unesterified D-glucose (n = 4 in all cases).

Figure 5 illustrates the effects of hexose esters and unesterified D-glucose on glucagon secretion. The first effect of the glucose esters was to provoke a rapid but short-lived stimulation of glucagon release. In terms of the absolute or relative magnitude of this early increment in glucagon secretion and its time course, there was little to distinguish between α-D-glucose pentaacetate and β-L-glucose pentaacetate. For instance, the peak values recorded at min 30–31 were 0.78 ± 0.26 and 0.56 ± 0.22 ng/min higher than the paired reading at min 28 in the case of α-D-glucose pentaacetate and β-L-glucose pentaacetate, respectively. Likewise, when expressed relative to the paired mean basal value (min 20–25), the mean output of glucagon during the early peak-shaped response (min 29–34) averaged 103.4 ± 7.6 and 115.4 ± 14.0% in pancreases exposed to α-D-glucose pentaacetate and β-L-glucose pentaacetate, respectively (Fig. 6).

The early stimulation of glucagon output by the glucose esters contrasted with an early inhibitory action of unesterified D-glucose on glucagon secretion. Indeed, as computed over two successive periods of 7 min each, the exponential line relating glucagon release (R) to time (t), according to the equation $R = R_0 \cdot e^{Kt}$, yielded a higher K value (P < 0.05) after (min 28–34) than before (min 22–28) introduction of the hexose, with a mean paired difference of 2.45 ± 0.67 × 10−²/min (n = 4).

The effects of α-D-glucose pentaacetate and β-L-glucose pentaacetate on glucagon release differed vastly from one another, however, from the 35th min onward. In pancreases exposed to the ester of L-glucose, the rate of glucagon release resumed a value virtually identical to that calculated by exponential extrapolation between the readings recorded at min 28 and 63, i.e., just before administration of the ester and 10 min after such an administration. Thus the mean output of glucagon between the 39th and 53rd min averaged 101.2 ± 3.4%
DISCUSSION

The present experiments were aimed at comparing the effects of \(\alpha\)-D-glucose pentaacetate, \(\beta\)-L-glucose pentaacetate, and unesterified D-glucose on insulin, somatostatin, and glucagon release by the isolated perfused rat pancreas. They were conducted in the presence of L-leucine (10.0 mM) to increase the magnitude of the insulin secretory response to the two esters of glucose (18). The esters were tested at a 1.7 mM concentration, which is close to their limit of solubility. No sizable hydrolysis of the esters took place in the perfusate.

The findings illustrated in Figs. 3 and 4 confirm that \(\alpha\)-D-glucose pentaacetate and, to a much lesser extent, \(\beta\)-D-glucose pentaacetate stimulate insulin release under experimental conditions in which unesterified D-glucose, tested at the same molar concentration, fails to do so. As already mentioned in the introduction, the insulinotropic action of the hexose esters cannot be attributed to the catabolism of their acetate moieties. Likewise, in the case of \(\beta\)-L-glucose pentaacetate, the stimulation of insulin release cannot be due to the catabolism of the hexose moiety of the ester, since L-glucose is not phosphorylated by hexokinase isoenzymes (unpublished observation). The secretory response to this ester thus appears unrelated to its nutritional value and, instead, could be due to a direct effect of the ester itself on some as-of-yet unidentified receptor system.

In addition to its vastly lower magnitude, the secretory response of the B cells to \(\beta\)-L-glucose pentaacetate differed from that evoked by \(\alpha\)-D-glucose pentaacetate by the virtual absence of an early secretory peak and the absence of a residual insulinotropic action when the administration of the ester was interrupted.

These two differences may well be attributable to the capacity of \(\alpha\)-D-glucose pentaacetate, as distinct from \(\beta\)-L-glucose pentaacetate, to generate D-glucose by intracellular hydrolysis of the ester with further phosphorylation and metabolism of this hexose in the islet cells (20). Such a metabolic component of the secretory response could indeed be required to allow for the occurrence of an early secretory peak. It may also account for the existence of a residual stimulation of insulin release after removal of the ester from the perfusate. Indeed, the large amounts of the ester that accumulate in islet cells (20) may be sufficient to maintain for some time a sizable glycolytic flux.

Many of the considerations so far presented may also apply to the secretion of somatostatin. Such is the case, for instance, for the difference between \(\alpha\)-D-glucose pentaacetate and \(\beta\)-L-glucose pentaacetate in terms of the magnitude of the secretory response to these esters and their residual effect on hormonal output when removed from the perfusate. Likewise, both the insulin and somatostatin release evoked by unesterified D-glucose at the end of the experiments was somewhat higher after prior exposure to D-glucose as compared with \(\beta\)-L-glucose pentaacetate and unesterified D-glucose, respectively. It was thus somewhat more pronounced (\(P < 0.05\)) in the first case than in the latter two.
cells. This view is further supported by the fact that, relative to the mean value found with α-D-glucose pentaacetate, the steady-state secretory rate found between the 46th and 50th min of exposure to β-L-glucose pentaacetate was lower, whether expressed in absolute terms or relative to basal output, in the case of insulin than somatostatin release, with overall mean values of 8.0 ± 1.6 and 41.2 ± 12.4% (P < 0.02).

The most dramatic evidence for the postulated dual mode of action of the glucose esters in the endocrine pancreas was provided by the measurements of glucagon release. Thus the two esters first stimulated glucagon secretion in a comparable manner, whether in terms of the magnitude or time course of this early secretory response, suggesting that such a glucagonotropic action is unrelated to the nutritional value of the esters. However, the two series of experiments differed from one another by the fact that α-D-glucose pentaacetate, but not β-L-glucose pentaacetate, inhibited glucagon release during the late period of exposure to the esters, this effect being reversed after removal of α-D-glucose pentaacetate from the perfusate. Likewise, α-D-glucose pentaacetate, but not β-L-glucose pentaacetate, apparently primed the glucagon-producing cells to the inhibitory action of unesterified D-glucose administered at the end of the experiments. The latter two phenomena are likely to result from the metabolism of the hexose moiety of α-D-glucose pentaacetate, since they indeed duplicate the effect of unesterified D-glucose on the secretory behavior of A cells. The inhibitory action of the hexose on glucagon release is indeed well known. Likewise, several reports have already documented that prior exposure of the pancreas to d-glucose decreases the secretory response of α-β-cells to the subsequent administration of arginine (3, 4). To our knowledge, however, the results obtained in the experiments conducted with α-D-glucose pentaacetate provide the first evidence for A cell priming in terms of the inhibitory action of D-glucose on glucagon output.

It should also be stressed that in the present experiments, unesterified D-glucose, when tested at the low concentration of 1.7 mM, obviously inhibited glucagon release despite failing to cause any sizable increase in insulin release. This finding is compatible with the view that the hexose can inhibit glucagon secretion independently of any stimulation of insulin secretion (9).

In conclusion, the present findings convincingly document that glucose esters, such as α-D-glucose pentaacetate and β-L-glucose pentaacetate, affect insulin release through a dual mode of action and extend such a knowledge to the secretion of both somatostatin and glucagon by the endocrine pancreas. In our opinion, advantage could be taken of the fact that esters of nonmetabolizable hexoses stimulate insulin release by an as-yet unknown mechanism, to develop new insulinotropic tools for the treatment of non-insulin-dependent diabetes (12, 14).

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