Reviews

On the track to the beta-cell*

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Abstract

The imaging and quantification of the endocrine pancreas by a non-invasive procedure remains a challenge. In the prolongation of prior work on selected succinic acid esters and monosaccharide esters, it is proposed that D-mannoheptulose, which might be transported across the plasma membrane at the intervention of GLUT-2, could be used to label preferentially the endocrine moiety of the pancreatic gland. This heptose is taken up more efficiently by hepatocytes and islet cells, than by erythrocytes, parotid cells, acinar pancreatic cells or tumoural islet cells of either the RINm5F or INS-1 line. Likewise, D-mannoheptulose only inhibits D-glucose metabolism in hepatocytes and isolated islets. Its hexaacetate ester, however, inhibits the catabolism of the hexose in all cell types. The uptake of D-mannoheptulose repre-

sents a carrier-mediated process. Human islets behave like rat islets in terms of D-mannoheptulose uptake and inhibition by the heptose of both D-glucose metabolism and insulinotropic action. The use of radiolabelled analogs of D-mannoheptulose suitable for imaging of the endocrine pancreas is discussed. In the same perspective, it is proposed that advantage could be taken of the much greater accumulation of glycogen in insulin-producing cells, as compared to other pancreatic cell types, in situations of sustained hyperglycaemia. [Diabetologia (2001) 44: 393–406]

Keywords Beta-cell imaging, succinic acid esters, monosaccharide esters, D-mannoheptulose, human islets, islet transplantation, 6-deoxy-6-iodo-D-glucose, alloxan, glycogen, 2-deoxy-2-fluoro-D-glucose, positron emission tomography.

In his famous article published in the *Lancet* in 1969 and entitled “Why are the islets of Langerhans”, John Henderson wrote: “A hundred years ago Paul Langerhans described, in the most modest way, some pale patches of cells that he had noticed in the pancreas of the rabbit. For the past fifty years these pale patches have been the subject of a steady stream of human curiosity: they have been stained, sliced, given to patients to eat, counted, labelled, stimulated, ex-

tracted, isolated, and exhausted. Yet it is far from clear why they should exist in this form at all” [1]. One could add: ‘yet, it is still not possible to visualize and quantify the islets of Langerhans in the intact organism by a non-invasive method’. The main topics of this presentation are to review the difficulties encountered in the non-invasive imaging of the endocrine pancreas and to discuss two approaches that could conceivably be followed to achieve such a goal.

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Background information

The background information which led us to consider this issue can be summarized as follows.

In the beta-cell of non-insulin-dependent diabetic subjects, a preferential impairment of the secretory

Fig. 1. Schematic view of the beta-cell in Type II diabetes, with emphasis on the impairment of D-glucose catabolism and the possible use of non-glucidic nutrients to bypass the defect in hexose utilization

response to D-glucose, as distinct from other nutrient or non-nutrient secretagogues, may be attributable to a site-specific defect in D-glucose transport [2, 3], phosphorylation [4] or further catabolism so that not enough ATP is generated to provoke the otherwise expected closing of ATP-sensitive K^+ channels [5] (Fig. 1).

In addition, non-glucidic nutrients able to bypass the anomaly in D-glucose utilization could, as a result of their efficient metabolism in the beta-cell, restore a high rate of ATP generation and, hence, restore the closed state of the K^+ channels. The emphasis here is on the esters of succinic acid for two reasons. First, succinic acid, being a metabolite of the Krebs cycle, would indeed be expected to bypass the postulated deficiency in D-glucose transport, phosphorylation or further catabolism. Second, and as first documented in hepatocytes [6], esters of succinic acid, such as its monomethyl or dimethyl ester, penetrate easily into cells in contrast to succinic acid itself which is virtually unable to enter into islet cells and is devoid of any insulintropic action [7, 8].

One among other objections that we duly formulated when considering this new therapeutic approach consists in the undesirable generation of methanol resulting from the intracellular hydrolysis of monomethyl or dimethyl succinic acid [9].

To overcome this objection, we then developed a number of other esters of succinic acid including esters in which a carboxylic group of succinic acid was esterified by the alcohol function on the carbon 6 of selected monosaccharides. These esters were found to stimulate insulin release both in vitro and in vivo

Fig. 2. Optical density of formozan in RINm5F cells cultured for 1 to 4 days in the presence of 2-deoxy-D-glucose tetraacetate (2-DOGTA; 10.0 μ mol/l), α -difluoromethylornithine (α -DFMO; 10.0 μ mol/l) or both compounds (each 10.0 μ mol/l). Mean values ($\pm$ SEM) are expressed relative to the mean control value found at the same time in cells cultured in the absence of any antitumoral agent

[10, 11]. It should be emphasized that, in these experiments, we were careful to select monosaccharides, such as D-galactose or 3-O-methyl-D-glucose, known to be devoid of insulintropic action, so that the stimulation of insulin release by these esters could be safely attributed to the catabolism of their succinate moiety rather than carbohydrate moiety.

We later achieved exactly the opposite goal using monosaccharide esters, such as α -D-glucose pentaacetate, which were formed of a well-known insulintropic hexose, in this case α -D-glucose, and an acid, in this case acetic acid, devoid of any statistically significant insulin-releasing capacity. Once again, the selection of these monosaccharide esters was motivated by the idea that they would probably enter into cells without requiring the intervention of a specific carrier system and then undergo esterase-catalysed intracellular hydrolysis, so that their carbohydrate moiety would then become readily available for phosphorylation and further catabolism [12]. This working hypothesis was soon validated [13] and our work with

Fig. 3. D-glucose metabolism in different cell types. Mean results ($\pm$ SEM) recorded in the presence of either D-mannoheptulose (1.0–10.0 mmol/l; upper panel) or its hexaacetate ester (2.0–5.0 mmol/l; lower panel) are expressed relative to the mean corresponding control values

such monosaccharide esters led to several series of contributions, three of which are mentioned below.

First, α -D-glucose pentaacetate does indeed bypass defects of glucose transport across the plasma membrane. For instance, in experiments conducted in three types of muscles obtained from either control mice or GLUT-4 deficient mice, the incorporation of D-[U- 14 C]glucose into glycogen was invariably impaired in the GLUT-4 deficient animals whether in diaphragm, soleus or extensor digitorum longus. No significant difference in glycogen labelling was, however, observed anymore, when α -D-[U- 14 C]glucose pentaacetate was used as the exogenous nutrient [14].

Second, in the same manner that the esterification of metabolizable monosaccharides augments their nutritional value and insulinotropic capacity, the esterification of certain non-metabolizable monosaccharides could considerably augment their efficiency as inhibitors of glucose catabolism [15, 16]. We took advantage of the latter situation to document that in several lines of tumoral cells 2-deoxy-D-glucose tetra-

Fig. 4. The intracellular distribution space of D-[5- 3 H]glucose (vertically hatched rectangles) and D-[3 H]mannoheptulose (black rectangles, corresponding to the mean values $\pm$ SEM) and the [U- 14 C]sucrose space (horizontally hatched rectangles shown below the zero ordinates), taken as the extracellular volume, are all expressed relative to the intracellular 3 HOH space found in the same cell type

acetate is much more potent than unesterified 2-deoxy-D-glucose as an antitumoral agent [17, 18]. The ester is also quite potent in enhancing the antitumoral action of established chemotherapeutic agents [19]. For instance, 2-deoxy-D-glucose tetraacetate tested at a low concentration of 10 μ mol/l, insufficient to affect the growth of RINm5F cells, caused a shift to the left of the time-related response to α -difluoromethylornithine also tested at a 10 μ mol/l concentration (Fig. 2). These in vitro findings were then extended to in vivo experiments, for example in mice inoculated with ascites-producing tumoral cells [20]. Comparable findings were also made in several lines of human tumoral cells [18, 21, 22] and clinical trials are now in progress to assess the value of 2-deoxy-D-glucose tetraacetate as an antitumoral agent, for instance in melanoma patients.

Third, in a comparable manner, we observed that the esterification of D-mannoheptulose, a well-known inhibitor of D-glucose phosphorylation [23], confers to this heptose the capacity to inhibit D-glucose metabolism in cells otherwise resistant to unesterified D-mannoheptulose [24]. Parotid cells, erythrocytes and acinar pancreatic cells are totally resistant to unesterified D-mannoheptulose, the heptose having no statistically significant effect upon the generation

Fig. 5. Mean values ($\pm$ SEM) for ^3H fractional outflow rate from islets prelabelled with D- ^3H mannoheptulose and perfused in the presence or absence of cytochalasin B

of radioactive metabolites from tritiated or ^{14}C -labelled D-glucose, all results being expressed relative to the mean control value found in the absence of D-mannoheptulose [24–26] (Fig. 3). In sharp contrast to these findings, unesterified D-mannoheptulose significantly inhibits D-glucose metabolism in hepatocytes or isolated pancreatic islets [27–29]. Yet, in cell homogenates, D-mannoheptulose inhibits glucose phosphorylation in all these cell types [30, 31]. Moreover, the hexaacetate ester of D-mannoheptulose tested at a concentration equal to or lower than that of unesterified D-mannoheptulose also significantly inhibits D-glucose metabolism in all these cell types [24–26, 28, 29] (Fig. 3).

Working hypothesis

Since only hepatocytes and islet beta-cells are equipped with GLUT-2, a possible explanation for these findings could be that unesterified D-mannoheptulose is transported into cells at the intervention of this specific carrier system. If so, advantage could conceivably be taken from the contrasting situation found in

^3H -mannoheptulose phosphorylation by purified hexokinase isoenzymes

Fig. 6. Mean values ($\pm$ SEM) for D- ^3H mannoheptulose phosphorylation by bovine heart hexokinase (left) and human beta-cell glucokinase (right) at increasing concentrations of D-glucose

acinar versus islet cells to label preferentially the endocrine moiety of the pancreatic gland with radioactive D-mannoheptulose. This could thus allow to quantify the total mass of insulin-producing cells in the pancreas.

This working hypothesis was recently formulated in an abstract presented at the June (2000) meeting of the American Diabetes Association [32] and entitled “GLUT-2 mediated transport of D-mannoheptulose: a tool for imaging of the endocrine pancreas?”

D-mannoheptulose uptake and phosphorylation by isolated cells

In order to assess the validity of this proposal, we have first measured the uptake of tritiated D-mannoheptulose by different cell types, the cells being separated after 30 to 60 min incubation at 37°C from their surrounding medium by centrifugation through a layer of oil [33]. All results in Figure 4 are expressed relative to the intracellular water space, taken as the paired difference between the distribution spaces of ^3HOH and $[\text{U-}^{14}\text{C}]\text{sucrose}$. The horizontally hatched rectangles shown below the zero ordinates document the values of the $[\text{U-}^{14}\text{C}]\text{sucrose}$ space in the different cell types. The vertically hatched rectangles shown above the zero ordinates represent the intracellular distribution space of D- ^3H glucose. Such an intracellular D- ^3H glucose space represented about half of the total intracellular water space. The black rectangles refer to the mean values ($\pm$ SEM) for the intracellular distribution space of tritiated D-manno-

Fig. 7. Radioactive content of islets transplanted in an implantation device, liver and pancreas in a streptozotocin-induced diabetic rat injected with D-[³H]mannoheptulose 24 h before killing. Results are expressed per μg DNA and ranged on a logarithmic scale. The inset refers to results expressed per mg wet weight and also ranged on a logarithmic scale. The horizontal dotted line emphasizes that the radioactive content of the transplanted islets was about eight times higher and that of the pancreas about one third lower than that of plasma

heptulose. It did not exceed about 1 and 12 % of the intracellular ³HOH space in rat erythrocytes and parotid cells, respectively. These two examples are representative of cell types in which D-mannoheptulose fails to affect the catabolism of D-glucose. The distribution space of tritiated D-mannoheptulose showed much higher values close to 45 to 50 % of the intracellular water space in hepatocytes, intact isolated islets and dispersed islet cells. In these instances, which refer to cells in which D-mannoheptulose indeed inhibits D-glucose catabolism, the distribution space of the tritiated heptose was close to that of D-[5-³H]glucose. These results contrasted with those found in tumoral islet cells of either the RINm5F or the INS-1 line. In these cells, D-mannoheptulose virtually fails to affect D-glucose metabolism and the distribution space of tritiated D-mannoheptulose again did not exceed about 3 to 10 % of the intracellular water space, a situation comparable to that found in erythrocytes or parotid cells. It should be emphasized, however, that whilst no statistically significant difference could be found between RINm5F and INS-1 cells in terms of

the absolute values for the distribution spaces of tritiated water, [U-¹⁴C]sucrose or D-[5-³H]glucose, the intracellular distribution space of tritiated D-mannoheptulose was three times higher in INS-1 cells than in RINm5F cells. The latter difference was highly statistically significant and represented the sole detectable difference between the two types of tumoral islet cells. It coincides with the fact that the expression of GLUT-2 predominates over that of GLUT-1 in INS-1 cells, albeit not so in RINm5F cells [34, 35]. Taken as a whole, therefore, these data are compatible with the view that the entry of D-mannoheptulose into cells is mediated mainly, if not exclusively, by GLUT-2.

Further experiments conducted in isolated islets and dispersed islet cells indicated, in fair agreement with [36], that the uptake of tritiated D-mannoheptulose represents a carrier-mediated process, it being time-related over 3 to 120 min incubation at 37°C, much lower at 4 than at 37°C and inhibited by cytochalasin B. It was enhanced, in a time-dependent manner, by 8.3 mmol/l D-glucose and was much lower than the uptake of D-[³H]mannoheptulose hexaacetate, as well as in erythrocytes, parotid cells, hepatocytes and pancreatic acinar cells [33, 37]. It was associated with the accumulation of tritiated acidic metabolites in the islet cells.

The inhibition of D-mannoheptulose uptake by cytochalasin B coincided with a lesser inhibition by the heptose of D-glucose metabolism in rat islets. Thus, the conversion of both D-[5-³H]glucose and D-[U-¹⁴C]glucose to their respective metabolites was, relative to the paired control value found in the absence of D-mannoheptulose, less severely affected by the heptose in the presence of cytochalasin B than in its absence [38].

In islets prelabelled with tritiated D-mannoheptulose and perfused in the presence of 20 μmol/l cytochalasin B, the removal of the mould metabolite from the perfusate provoked a rapid, sustained and rapidly reversible increase in effluent radioactivity, indicating that cytochalasin B also inhibits the efflux, as well as the influx, of the heptose across the plasma membrane (Fig. 5).

The generation of tritiated acidic metabolites in cells exposed to D-[³H]mannoheptulose was found attributable to phosphorylation of the heptose, as documented in experiments conducted with either purified hexokinase isoenzymes or crude cell homogenates [33]. In these experiments, the phosphate ester of D-[³H]mannoheptulose was separated from its precursor by anion-exchange chromatography. Increasing concentrations of D-glucose in the 0.1 to 1.0 mmol/l range cause a concentration related inhibition of D-mannoheptulose phosphorylation by bovine heart hexokinase, such an inhibitory effect being of the competitive type (Fig. 6, left panel). An opposite situation prevails in the case of human beta-cell glucokinase, D-glucose in the 1 to 20 mmol/l range

Fig. 8. Tridimensional view (top) and skeleton (bottom) of D-glucose (left) and 6-deoxy-6-iodo-D-glucose (right)

now causing a bell-shaped increase in D-mannoheptulose phosphorylation (Fig. 6, right panel). The latter situation is virtually superimposable to that recently identified in our laboratory with another ketose, namely D-fructose [39]. It might account for the effect of D-glucose to increase the net uptake of tritiated D-mannoheptulose by islet cells. Moreover, it opens the perspective that the non-invasive imaging of the endocrine pancreas could be facilitated by measurements made at a relatively late time after injection of the tracer molecule, when its concentration in the bloodstream would become negligible relative to the islet cell content in radioactive acidic metabolites, which remain inside dispersed islet cells even after extensive and prolonged washing.

D-mannoheptulose uptake in vivo by pancreatic islet and acinar cells

With this information in mind, we then attempted to directly compare in vivo the uptake of tritiated D-mannoheptulose by pancreatic islet and acinar cells. Mice were injected with streptozotocin and then transplanted a week later under the left kidney capsule with groups of 300 to 550 islets each obtained from animals of the same strain. The animals were injected intravenously with tritiated D-mannoheptulose 7 to 14 days after islet transplantation, and 60 min lat-

6-deoxy-6- $[^{125}\text{I}]$ iodo-D-glucose intravenous injection

Fig. 9. Mean values ($\pm$ SEM) for plasma D-glucose concentration and paired pancreas to blood radioactive ratio in control rats and insulin-treated or untreated streptozotocin-induced diabetic animals examined 3 min after intravenous injection of 6-deoxy-6- $[^{125}\text{I}]$ iodo-D-glucose. The number of individual observations is shown at the bottom of each column

er samples of plasma, liver and pancreas, and a fragment of the kidney capsule with the transplanted islets were examined for their radioactive content [40]. Relative to either wet weight or DNA content, the radioactive content of the liver and transplanted islets was five to eight times higher than the paired value found in the pancreatic gland. Incidentally, the results obtained in the transplanted islets were comparable when corrected for the measurements made in a sample of the contralateral kidney.

Essentially comparable results were obtained in streptozotocin-induced diabetic rats transplanted with 1 to 4 groups of 1500 islets each placed in an implantation device that was inserted in the peritoneal cavity [41]. Thus, 24 hours after the intraperitoneal injection of D- $[^3\text{H}]$ mannoheptulose, the radioactive content of the transplanted islets, expressed relative to the mean wet weight of the isolated rat islets (5.0 μg) was much higher than that found in the pan-

Assessment of total islet beta-cell mass in the isolated perfused pancreas

Fig. 10. Assessment of total islet beta-cell mass in the isolated perfused pancreas. D-[^3H]mannoheptulose (0.1 mmol/l) and [^{14}C]sucrose (0.5 mmol/l) were administered from min 30 to 45 and cytochalasin B (20 $\mu\text{mol/l}$) from min 40 to 55. The lower left panel illustrates the ^3H outflow, expressed relative to the mean reference value recorded between min 41 to 45 inclusive. The right panel provides the mean values ($\pm$ SEM) for the intracellular D-[^3H]mannoheptulose space in control rats (C) and streptozotocin-induced diabetic rats (STZ)

creatic gland or other tissues, such as muscle, kidney, plasma and even liver. Moreover, even when expressed relative to their measured DNA content, the radioactive content of the transplanted islets remained about five times higher than that found in the pancreas, despite that acinar cells are sizeably larger than islet cells (Fig. 7).

These experiments confirm that the uptake of tritiated D-mannoheptulose is much higher in hepatocytes or islet cells than in acinar pancreatic cells and, hence, support the proposal that the heptose could conceivably be used to label preferentially the endocrine moiety of the pancreatic gland.

Potential objections

At least two objections could be formulated, however, against such a proposal. The first possible objection is inspired by the knowledge that in human pancreatic islets, GLUT-1, rather than GLUT-2 represents the major carrier system for the transport of monosaccharides across the beta-cell plasma membrane [42, 43].

Pancreatic [$2\text{-}^{14}\text{C}$]alloxan uptake in vitro and in vivo

Fig. 11. Mean values ($\pm$ SEM) for [$2\text{-}^{14}\text{C}$]alloxan uptake by pancreatic pieces from control and streptozotocin-induced diabetic rats incubated for 10 to 60 min at 37°C (upper panel) and for pancreatic radioactive content in control and insulin-treated streptozotocin-induced diabetic rats examined 60 or 1440 min after intravenous injection of [$2\text{-}^{14}\text{C}$]alloxan (lower panel). The data mentioned in each brace refer to the overall mean values, expressed relative to the measurements made at the same time in control rats, and emphasize the close quantitative analogy between the results of in vitro and in vivo experiments

The following findings argue, however, against such a potential objection [44].

First, in human islets, D-mannoheptulose, tested at a 10 mmol/l concentration, as well as its hexaacetate ester used at a 5 mmol/l concentration, both inhibit insulin release evoked by 16.7 mmol/l D-glucose over 60 min incubation at 37°C .

Second, in human islets, D-mannoheptulose, again tested at a 10 mmol/l concentration, inhibits both the conversion of D-[$5\text{-}^3\text{H}$]glucose to ^3HOH and that of D-[^{14}C]glucose to $^{14}\text{CO}_2$ over 120 min incubation in the presence of 11.1 mmol/l D-glucose.

Radioactive glycogen content of the pancreas

Fig. 12. Mean values ($\pm$ SEM) for the pancreatic content in ^{14}C -labelled glycogen (expressed as D-[U- ^{14}C]glucose equivalent, by reference to the specific radioactivity of the infused hexose) and insulin (inset) in control rats (C) and streptozotocin-induced diabetic animals (STZ) infused for 48 h with a hypertonic solution of D-glucose (1.67 mol/l in control rats and 0.25 mmol/l in STZ rats)

Third, in human islets, the apparent distribution space of tritiated D-mannoheptulose, always tested at a 10 mmol/l concentration, largely exceeds that of uniformly [U- ^{14}C]sucrose, used as an extracellular marker, and actually is not significantly different from the total distribution space of tritiated water.

Our second concern refers to the selection of a suitably labelled D-mannoheptulose analogue in the perspective of the non-invasive imaging of the endocrine pancreas. Three possible candidates for such a role include ^{11}C -labelled D-mannoheptulose, 3-deoxy-3-[^{18}F]fluoro-D-mannoheptulose and 7-deoxy-7-[^{123}I]iodo-D-mannoheptulose. The two first of these candidates are mentioned here by analogy with the

Pancreatic glycogen content in STZ and glucose-infused rats

Fig. 13. Mean values ($\pm$ SEM) for pancreatic glycogen content in streptozotocin-induced diabetic rats (STZ) and control animals infused with D-glucose (1.67 mol/l) for 2 to 3 days (open columns). The glycogen content of the exocrine (acini) and endocrine (islets) moieties of the pancreatic gland (black columns) was judged, respectively, from the results recorded in STZ rats and the difference between the measurements made in the two types of rats, assuming that islets account for one percent of the total pancreatic mass

current use of ^{11}C -labelled D-glucose or 2-deoxy-2-[^{18}F]fluoro-D-glucose for imaging of several organs by positron emission tomography. I will return to this matter later in this report and just mention at this point that, with the help of chemists working in our laboratory and at New England Nuclear in Boston, we have now been able (1) to devise a method for the synthesis of [1- ^{14}C]labelled D-mannoheptulose which is sufficiently rapid to be applied to the preparation of ^{11}C -labelled D-mannoheptulose, despite the short half-life of ^{11}C , and (2) to achieve the synthesis of 3-deoxy-3-fluoro-D-mannoheptulose. The third candidate mentioned above offers the advantages of using an isotope with a longer half-life and suitable for imaging by single photon emission computerized tomography. Because of the high mass of the iodine atom, iodinated D-mannoheptulose may not, however, show the same specificity towards GLUT-2 as the non-iodinated heptulose.

^{18}F and ^{14}C glycogen labelling in islets cultivated at 30 mmol/l D-glucose

Fig. 14. Mean values ($\pm$ SEM) for the radioactive glycogen content (pmol of D-glucose equivalent per islet) of islets cultured for 2 days at 30 mmol/l D-glucose, a tracer amount of either 2-deoxy-2- ^{18}F -fluoro-D-glucose or D- ^{14}C -glucose being present in the culture medium during the last 60 min. The inset illustrates the time course for radioactive glycogen accumulation in islets cultured at 30 mmol/l D-glucose in the presence of D- ^{14}C -glucose

This issue is illustrated in Fig. 8, which refers to results obtained by conformational analysis and comparing the skeleton and the tridimensional view of D-glucose to the left and 6-deoxy-6-iodo-D-glucose to the right. The figure shows the voluminous mass of the iodine atom. Despite these steric differences, previous work has documented that this molecule of 6-deoxy-6-iodo-D-glucose can be used in vitro or in vivo as a tracer for GLUT-mediated D-glucose transport in different cell types [45]. We have recently extended such findings, with emphasis on the uptake of the same molecule by the pancreas.

We observed that, in vitro, the uptake of 6-deoxy-6- ^{125}I -iodo-D-glucose reaches a close-to-steady state value much earlier in pancreatic islets than in pieces of pancreas [46]. A similar situation was documented using D- ^{14}C -glucose [26].

We took advantage of such a vastly different time course for the uptake of D-glucose by acinar versus islet pancreatic cells to achieve a preferential labelling of the endocrine pancreas in vivo 3 min after the intravenous injection of 6-deoxy-6- ^{125}I -iodo-D-glucose [47].

The plasma D-glucose concentration was virtually identical in control rats and streptozotocin-induced

Fate of 2-deoxy-2- ^{18}F -fluoro-D-glucose in normal rats

Fig. 15. Mean values ($\pm$ SEM) for the radioactive content of several tissues, relative to the paired plasma measurement, in normal rats examined 3 min, 15 min and 240 min after intravenous injection of 2-deoxy-2- ^{18}F -fluoro-D-glucose

diabetic rats treated with insulin and much lower than that found in untreated streptozotocin animals (Fig. 9). The radioactive content (lower panel) of the pancreatic gland, expressed relative to the paired blood value, was not different in the insulin-treated or non-treated streptozotocin rats but, in both cases, significantly lower than the control value.

Thus, even with a monosaccharide, namely D-glucose, which, at variance with D-mannoheptulose, is not specifically taken up by endocrine pancreatic cells, it is possible to document an obvious difference in the uptake of the radioactive tracer by the whole pancreatic gland, as attributable to the presence or absence of insulin-producing cells by comparing, under suitable experimental conditions, the situation in normal rats and that in STZ-induced diabetic animals.

As soon as the radioactive analogues of D-mannoheptulose already mentioned will be made available, we will conduct the pilot in vivo tests to eventually compare the pancreatic uptake of these analogues in

Fig. 16. Comparison between the radioactive signals recorded in coronal, transaxial and sagittal sections 60 min after injection of 2-deoxy-2-[¹⁸F]fluoro-D-glucose and later after injection of ¹¹C-labelled methionine in a normal volunteer infused with a hypertonic solution of D-glucose

normal versus Type I diabetic animals and human subjects.

Possible practical application

At present, a practical application of this knowledge consists in the assessment of the total islet beta-cell mass by measurement of the radioactive content of the whole pancreas after perfusion of isolated pancreatic glands with D-[³H]mannoheptulose (Fig. 10). After a 30 min equilibration period, the pancreas is first perfused with tritiated mannoheptulose to label preferentially the insulin-producing cells and [U-¹⁴C]sucrose used as an extracellular marker. It is then perfused with a non-radioactive medium after and during administration of cytochalasin B (20 μmol/l) in order to wash out extracellular radioactivity whilst avoiding the release of the radioactive

heptose from the islet cells. Under these experimental conditions, the intracellular distribution space of ³H-mannoheptulose is not significantly different from zero in streptozotocin animals, whilst representing about 0.6% of the total pancreatic mass in control animals. This procedure thus allows to assess the total beta-cell mass without requiring painstaking morphometric analysis. We have now designed a comparable procedure to assess the relative contribution of beta-cells to the total mass of isolated islets.

The fact that the islets of Langerhans, despite representing only 1% of the total pancreatic mass, could contribute to a sizeable extent to the uptake of suitable tracers by the whole pancreatic gland was further documented in experiments dealing with the uptake of [2-¹⁴C]alloxan by the pancreas from control or streptozotocin-induced diabetic rats [48]. The uptake of alloxan by pieces of pancreas from streptozotocin rats only represented after 10 to 60 min incubation 68% of the control value found in normal rats (Fig. 11). Likewise, 1 h or 24 h after the intravenous injection of alloxan, the radioactive content of the pancreatic gland only represented in the streptozotocin-induced diabetic rats, which were treated with insulin to restore normoglycemia, 65% of the control value found in normal rats (Fig. 11).

Fig. 17. Mean values ($\pm$ SEM) for the liver and pancreas ^{14}C content, relative to the paired blood measurement, in normal rats infused with D-glucose (1.67 mol/l) for 100 min and injected with D-[U- ^{14}C]glucose at the 60th min of such an infusion. The inset illustrates the increase in plasma D-glucose and insulin concentrations resulting from the infusion of the hexose

Alternative tools for preferential labelling of the endocrine moiety of the pancreas

At this point, it should be mentioned that D-mannoheptulose might not be the sole tool for the preferential labelling of the endocrine pancreas. Other radioactive molecules, that were examined during the past year in our laboratory for such a purpose, include ^{65}Zn [49], tritiated glibenclamide [50], tritiated mitiglinide [51] and a ^{125}I -labelled mouse monoclonal antibody directed against pancreatic beta-cell surface ganglioside(s) [52].

Under the experimental conditions so far investigated, however, none of these radioactive tracers provided convincing results when their uptake by the pancreatic gland was compared in control versus STZ-induced diabetic rats, whether by in vitro or in vivo procedures.

Radioactive data in transplanted STZ rats infused with [U- ^{14}C]glucose

Fig. 18. Mean values ($\pm$ SEM) for the paired tissue to blood radioactive ratio (upper panel) and ^{14}C -labelled glycogen content (lower panel; logarithmic scale) in STZ-induced diabetic rats transplanted with 3 000 islets under the kidney capsule and later infused for 24 h with a 1.7 mol/l solution of D-glucose (mixed with a tracer amount of D-[U- ^{14}C]glucose)

Glycogen accumulation in islet beta cells

Another approach, however seems more promising. It takes advantage of the fact that, in situations of sustained hyperglycaemia, for instance in spontaneously diabetic animals, large amounts of glycogen accumulate in insulin-producing cells but apparently not in acinar cells [53–55].

In a way, this second approach represents a tribute to Claude Bernard, who discovered glycogen and in honour of whom this lecture is named.

We reasoned that, if glycogen could be labelled by a suitable radioactive tracer during protracted provoked hyperglycaemia, the radioactive content of the pancreas would possibly reflect the mass of its endocrine moiety.

Our pilot experiments in this perspective provided quite encouraging results (Fig. 12). They indicated that in rats infused for 48 h with a hypertonic solution of glucose mixed with a tracer amount of D-[U- ^{14}C]glucose, the radioactive glycogen content of the pancreas, expressed by reference to the specific radioactivity of infused D-glucose, was about 20 times higher in control animals than in streptozotocin-induced diabetic animals, virtually in proportion to the insulin content of the pancreas. Moreover, as judged

Glycogen accumulation in tumoral islet and acinar cells

Fig. 19. Mean values ($\pm$ SEM) for the radioactive glycogen content of RINm5F, INS-1 and AR42 J cells cultured for 72 h in the presence of 30 mmol/l D-glucose (mixed with a tracer amount of D-[U-¹⁴C]glucose). The inset illustrates the concentration dependency for glucose-induced glycogen accumulation in RINm5F cells (open circles), INS-1 cells (crosses) and isolated rat islets (closed circles) cultured for 48 h, the results being expressed as pmol of D-glucose equivalent per nl of intracellular water space

from the glycogen content of the pancreatic gland in streptozotocin-induced diabetic rats compared with control rats that became hyperglycaemic after infusion of D-glucose for 2 to 3 days (Fig. 13), the glycogen content per unit wet weight is about 75 times higher in islets than in acinar pancreatic cells [56].

Further work along this line of thinking aimed mainly at defining the optimal experimental conditions to label this pool of pancreatic glycogen with a tracer molecule such as ¹¹C-labelled D-glucose or 2-deoxy-2-[¹⁸F]fluoro-D-glucose suitable for measurements by a non-invasive imaging procedure. Thus, after characterization of the time course for glycogen accumulation in islets cultured at a 30 mmol/l concentration of D-glucose, as documented in the inset of Figure 14, we compared the labelling of islet glycogen by D-[U-¹⁴C]glucose versus 2-deoxy-2-[¹⁸F]fluoro-D-glucose after a prior culture period of 2 days at the same high glucose concentration [57]. As expected,

because 2-deoxy-2-fluoro-D-glucose 6-phosphate cannot be catabolised in the glycolytic pathway, the radioactive content of the glycogen pool was higher in the case of 2-deoxy-2-[¹⁸F]fluoro-D-glucose than D-[U-¹⁴C]glucose.

The time course for the radioactive labelling of several organs, including the pancreatic gland was then examined in normal rats after intravenous injection of 2-deoxy-2-[¹⁸F]fluoro-D-glucose [58]. The radioactive content of the pancreas was lower than the paired plasma or blood value 3 min and 15 min after the injection of the radioactive tracer (Fig. 15, upper two panels). As shown (Fig. 15, bottom panel), however, 4 h after the injection of 2-[¹⁸F]fluoro-2-deoxy-D-glucose, the pancreatic radioactive content represented three times the paired plasma or blood value. In rats infused with a hypertonic solution of D-glucose and examined as late as 8 h after the injection of 2-deoxy-2-[¹⁸F]fluoro-D-glucose, the radioactive content of the pancreas was close to four times the paired plasma value [59].

Unexpectedly, however, when comparable experiments were conducted in a few normal volunteers, virtually no detectable radioactive signal was produced by the pancreatic gland after injection of 2-deoxy-2-[¹⁸F]fluoro-D-glucose, despite the unambiguous localization of the pancreas achieved by a subsequent administration of ¹¹C-labelled methionine, as documented by comparison of the upper and lower panel in Figure 16. At variance with the pancreas, the liver, muscles and renal pelvis were well identified after injection of 2-deoxy-2-[¹⁸F]fluoro-D-glucose. In the light of these negative findings, we now intend to use ¹¹C-labelled D-glucose instead of 2-deoxy-2-[¹⁸F]fluoro-D-glucose as the tracer molecule.

This does not appear as an unrealistic approach for several reasons, only two of which will be briefly mentioned. First, when D-[U-¹⁴C]glucose was injected during the infusion of unlabelled glucose to normal rats at a rate sufficient to cause over 100 min, a marked increase in plasma glucose and insulin concentrations, the paired pancreas to blood radioactive ratio remained well above unity, although being lower, than the corresponding liver to blood ratio (Fig. 17). It should be emphasized that, in these experiments, the animals were killed 40 min after the injection of D-[U-¹⁴C]glucose, this representing twice the half-life of ¹¹C.

Second, in STZ-induced diabetic rats transplanted with 3000 islets under the kidney capsule and later infused for 24 h with the 1.7 molar solution of D-glucose mixed with a tracer amount of [U-¹⁴C]glucose, the radioactive content of the liver and transplanted islets largely exceeded that of the pancreas (Fig. 18). Moreover, in these experiments, the radioactive glycogen content of the transplanted islets, although being about 10 times lower than that of the liver, was ap-

proximately 70 times higher than that of the pancreatic gland, in agreement with the non-radioactive data mentioned previously.

This alternative approach for imaging of insulin-producing cells also offers, over the use of a radioactive mannoheptulose analogue, the advantage of being apparently applicable to the localization of insulinoma in the pancreatic gland. Indeed, much larger amounts of radioactive glycogen accumulates in tumoral islet cells of the RINm5F or INS-1 cell lines than in tumoral acinar pancreatic cells of the AR42 J line, when cultured in the presence of uniformly ^{14}C -labelled glucose (Fig. 19). Moreover, in tumoral cells, as distinct from normal islet cells, the accumulation of large amounts of glycogen does not require exposure to an abnormally high glucose concentration (Fig. 19).

Concluding remarks

The information reviewed in this report unambiguously documents that it is possible to label preferentially the endocrine moiety of the pancreas, e.g. by radioactive D-mannoheptulose or even D-glucose. Under suitable experimental conditions, which require further experimental work to be optimized, the contribution of insulin-producing cells to the total pancreatic radioactive content could amount to one third or more, despite the fact that these cells represent no more than about one percent of the whole pancreatic mass. This suggests that imaging and quantification of these cells by a non-invasive procedure could become feasible in the not-too-distant future. In conclusion, therefore, we are indeed on the track to the beta-cell.

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