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REVIEW

Enzyme-to-Enzyme Channeling in the Early Steps of Glycolysis in Rat Pancreatic Islets

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The enzyme-to-enzyme channeling of metabolic intermediates is not an uncommon process. The present review draws attention to recent experimental work documenting, in rat pancreatic islets, the enzyme-to-enzyme channeling of α-D-glucose 6-phosphate between hexokinase isoenzyme(s), mainly glucokinase, and phosphoglucoisomerase. Likewise, the possible enzyme-to-enzyme channeling of β-D-fructose 6-phosphate between phosphoglucoisomerase and phosphofructokinase is briefly evoked. These considerations are relevant to the anomeric specificity of D-glucose metabolism, even in islets exposed to equilibrated D-glucose, to the perturbation of such an anomeric specificity in the phenomenon of so-called B-cell glucotoxicity, and to the correct interpretation of ^{3}HOH generation from D-[2-^{3}H]glucose.

Key Words: Enzyme-to-enzyme channeling; hexokinase isoenzymes; phosphoglucoisomerase; pancreatic islets; D-glucose anomers.

Introduction

The enzyme-to-enzyme channeling of metabolic intermediates represents a far-from-uncommon process. It consists in the direct transfer of a metabolite generated by a first enzyme to the catalytic site of the next enzyme in a given metabolic sequence, so that the metabolite under consideration does not reach a free pool of molecules. To cite only one example, enzyme-to-enzyme tunneling in the sequence of reactions catalyzed by succinyl-CoA synthetase, succinate dehydrogenase, and fumarase has been documented in several cell types including rat pancreatic islets *(1–5)*. The present review aims at drawing attention to a novel proposal concerning enzyme-to-enzyme channeling in the early steps of glycolysis in rat pancreatic islets.

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Working Hypothesis

Based on recent experimental data reviewed below, the proposal was made that α-D-glucose 6-phosphate undergoes enzyme-to-enzyme channeling between its site of formation at the level of hexokinase isoenzyme(s), mainly glucokinase *(6–9)*, and its site of conversion to β-D-fructose 6-phosphate at the phosphoglucoisomerase level. Likewise, β-D-fructose 6-phosphate may be channeled from phosphoglucoisomerase to phosphofructokinase.

Enzyme-to-Enzyme Channeling of α-D-Glucose 6-Phosphate

The metabolism of D-glucose is known to display anomeric specificity in rat pancreatic islets. For instance, the output of lactate is greater in islets exposed to α-D-glucose rather than β-D-glucose *(10)*. A potential objection to this situation consists in the extremely rapid interconversion of α- and β-D-glucose 6-phosphate, even more so that such an interconversion is further accelerated in the presence of phosphoglucoisomerase *(11)*. Hence, it could be argued that, because of such a rapid interconversion, no sizable difference in glycolytic flux would be expected to be found between islets exposed to α- vs β-D-glucose, except for that conceivably attributable to the difference in the phosphorylation of the D-glucose anomers at the hexokinase and/or glucokinase level. We have previously indicated that the latter difference, which is not denied, is not sufficient to account for the experimental results relative to the fate of α- and β-D-glucose in intact islets *(12)*. An alternative explanation for such experimental results could consist in the enzyme-to-enzyme channeling of α-D-glucose 6-phosphate between hexokinase isoenzyme(s) and phosphoglucoisomerase. The following findings are consistent with the latter proposal.

It was first observed that in rat pancreatic islets incubated for 60 min at 4°C in the presence of 8.3 m*M* of either α- or β-D-glucose, mixed with tracer amounts of the corresponding D-glucose anomer labeled with ^{3}H on either C_2 or C_5, the β/α ratio for ^{3}HOH production from D-[2-^{3}H]glucose (201.0 ± 72.4%; mean ± SEM in this and all following cases; d.f. = 58) largely exceeds that for ^{3}HOH production

from D-[5-^{3}H]glucose (39.4 ± 14.7%; d.f. = 50). Because the generation of ^{3}HOH from D-[2-^{3}H]glucose takes place mainly, but not exclusively, at the phosphoglucoisomerase level, these results indicate that the efficiency of ^{3}HOH production from D-[2-^{3}H]glucose relative to total glycolytic flux (as judged through the generation of ^{3}HOH from D-[5-^{3}H]glucose) is much higher in islets exposed to β-D-glucose rather than α-D-glucose *(6)*. Hence, these findings are compatible with the view that the enzyme-to-enzyme channeling of α-D-glucose 6-phosphate between hexokinase isoenzyme(s) and phosphoglucoisomerase and, possibly, that of β-D-fructose 6-phosphate between phosphoglucoisomerase and phosphofructokinase (see below) prevents numerous back-and-forth interconversions of the relevant hexose phosphates at the phosphoglucoisomerase level, as otherwise required to ensure their extensive detritiation *(13)*. Moreover, the experimental values mentioned above, as well as the ratio between the generation of ^{3}HOH from D-[2-^{3}H]glucose/D-[5-^{3}H]glucose, as recorded in islets incubated for 60 min at 4°C in the presence of 8.3 m*M* equilibrated D-glucose (46.2 ± 14.4%; d.f. = 66) could only be reached in a mathematical model for D-glucose catabolism in the islets involving enzyme-to-enzyme channeling of α-D-glucose 6-phosphate between hexokinase isoenzyme(s) and phosphoglucoisomerase *(6)*.

A second series of comparable experiments was then conducted in islets incubated at 2.8 m*M* D-glucose, instead of 8.3 m*M* D-glucose *(7)*. The selection of this low concentration of D-glucose aimed at minimizing the fractional contribution of glucokinase to the overall rate of D-glucose phosphorylation in the isolated islets. The salient difference between the first and second series of experiments consisted in the fact that, at the low concentration of D-glucose anomers (2.8 m*M*), the β/α ratio for ^{3}HOH production from D-[2-^{3}H]glucose (70.9 ± 12.6%; d.f. = 52) was no more significantly different from that for ^{3}HOH generation from D-[5-^{3}H]glucose (59.6 ± 12.4%; d.f. = 71). As confirmed by corresponding mathematical models for the catabolism of the D-glucose anomers, this indicates that a much larger fraction of α-D-glucose 6-phosphate generated at the level of the hexokinase isoenzyme(s) escapes from the process of enzyme-to-enzyme channeling in islets incubated at 2.8 m*M*, rather than 8.3 m*M*, α-D-glucose. Actually, in islets exposed to α-D-glucose, the fractional escape of α-D-glucose 6-phosphate from the channeling process, relative to its rate of formation, was increased from 1.8% at 8.3 m*M* D-glucose to 63.4% at 2.8 m*M* D-glucose, the latter percentage being about 35 times higher than the former one *(7)*. In this second series of experiments, like in the first one, the generation of ^{3}HOH from D-[2-^{3}H]glucose/D-[5-^{3}H]glucose, as recorded in islets incubated for 60 min at 4°C in the presence of 2.8 m*M* equilibrated D-glucose (39.7 ± 11.6%; d.f. = 39) was not significantly different from the theoretical value found in the corresponding mathematical model (Fig. 1). These findings strongly suggest that glucokinase

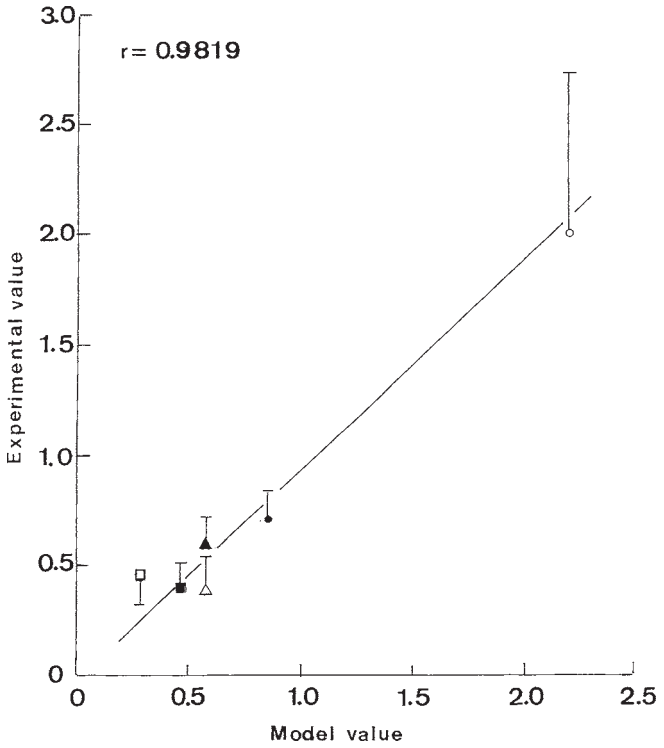

Fig. 1. Correlation between experimental values (mean ± SEM) and model values for the β/α ratio for ^{3}HOH generation from either D-[2-^{3}H]glucose (circles) or D-[5-^{3}H]glucose (triangles) by islets incubated for 60 min at 4°C in the sole presence of α-D-glucose or β-D-glucose and for the ratio between ^{3}HOH production from D-[2-^{3}H]glucose/D-[5-^{3}H]glucose (squares) by islets incubated in the presence of equilibrated D-glucose. Closed and open symbols refer to experiments conducted at a hexose concentration of 2.8 m*M* and 8.3 m*M*, respectively.

participates preferentially, relative to the low-K_m hexokinase, to the process of enzyme-to-enzyme channeling.

Such a view was further supported by experiments conducted in rat erythrocytes *(8)*. Indeed, in these cells, which are devoid of glucokinase, the regulation of the tunneling process by the extracellular concentration of D-glucose represented a mirror image of that observed in rat pancreatic islets. Thus, the β/α ratio for ^{3}HOH generation from D-[2-^{3}H]glucose by erythrocytes incubated for 60 min at 4°C in the sole presence of α- or β-D-glucose was not significantly different at 2.8 m*M* (117.4 ± 17.6%; d.f. = 37) and 8.3 m*M* (120.4 ± 27.4%; d.f. = 41), while the β/α ratio for ^{3}HOH production from D-[5-^{3}H]glucose was significantly lower ($p < 0.01$) at 2.8 m*M* (73.2 ± 10.2%; d.f. = 37) than at 8.3 m*M* (114.4 ± 11.2%; d.f. = 40).

In the last set of experiments, we measured the respective contributions of α-D-glucose and β-D-glucose to the overall generation of ^{3}HOH from D-[2-^{3}H]glucose or D-[5-^{3}H]glucose by islets incubated for 60 min at 4°C in the presence of 2.8 m*M* or 8.3 m*M* equilibrated D-glucose *(9)*. When expressed relative to the concentration of each anomer, the β/α ratios for ^{3}HOH production from D-[2-^{3}H]glu-

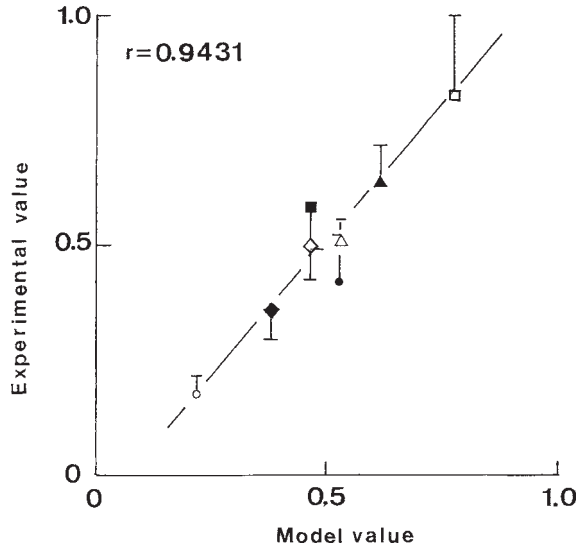

Fig. 2. Correlation between experimental values (means ± SEM) and model values for the fractional contribution of α-D-glucose (circles and triangles) and β-D-glucose (squares and diamonds) to the overall generation of ^{3}HOH from D-[2-^{3}H]glucose (circles and squares) or D-[5-^{3}H]glucose (triangles and diamonds) by islets incubated for 60 min at 4°C in the presence of 2.8 m*M* (closed symbols) or 8.3 m*M* (open symbols) equilibrated D-glucose.

cose or D-[5-^{3}H]glucose at the two concentrations of the hexose were close to those found in the two first series of experiments conducted in the sole presence of α- or β-D-glucose, with a correlation coefficient between these two sets of values of 0.9801 ($p < 0.02$). Thus, the anomeric specificity of hexose metabolism was still operative in islets exposed to equilibrated D-glucose, displaying the same pattern as that otherwise found in the sole presence of either α- or β-D-glucose. Moreover, as shown in Fig. 2, in this last set of experiments, the fractional contribution of each anomer to the overall generation of ^{3}HOH from equilibrated D-[2-^{3}H]glucose or D-[5-^{3}H]glucose, tested at 2.8 or 8.3 m*M*, was not significantly different from the corresponding theoretical value found in models for the catabolism of equilibrated D-glucose in rat pancreatic islets (Fig. 3).

Incidentally, it remains to be investigated whether β-D-glucose 6-phosphate may undergo enzyme-to-enzyme channeling between hexokinase isoenzyme(s) and the β-stereospecific enzyme glucose 6-phosphate dehydrogenase. In prior work, it was already documented that β-D-glucose is more efficiently metabolized in the pentose phosphate pathway than α-D-glucose. This was shown to be the case in both normal and tumoral pancreatic islet cells *(14)*.

Enzyme-to-Enzyme Channeling of β-D-Fructose 6-Phosphate

A most essential finding in the perspective of the present proposals resided in the demonstration that phosphoglucoisomerase, in addition to displaying anomeric specificity toward α-D-glucose 6-phosphate, also displays strict anomeric specificity toward β-D-fructose 6-phosphate *(10)*, at variance with prior assertions. This finding, documented by two-dimensional phase-sensitive ^{13}C exchange system nuclear magnetic resonance, allows a process of enzyme-to-enzyme channeling between phosphoglucoisomerase and phosphofructokinase to be visualized. The β-anomer of D-fructose 6-phosphate indeed represents the preferential substrate for phosphofructokinase. As a matter of fact, such a second process of enzyme-to-enzyme channeling was already considered in prior models for the catabolism of D-[2-^{3}H]glucose in pancreatic islets *(15)*. In these models, however, no reference was made to the anomeric specificity of such a catabolism. The latter issue was only considered in our more recent work on the fate of the anomers of D-glucose in pancreatic islets *(6,7)*. Nevertheless, it should be stressed that experimental evidence to support enzyme-to-enzyme channeling of D-fructose 6-phosphate between phosphoglucoisomerase and phosphofructokinase was already presented in a study conducted in cross-linked and permeabilized rat erythrocytes *(16)*.

Concluding Remarks

The present review concerns the enzyme-to-enzyme channeling of α-D-glucose 6-phosphate between hexokinase isoenzyme(s), mainly glucokinase, and phosphoglucoisomerase and that of β-D-fructose 6-phosphate between phosphoglucoisomerase and phosphofructokinase. The relevance of these processes to a number of biochemical or metabolic issues should not be overlooked.

First, the proposed channeling processes are essential to understand the anomeric specificity of D-glucose metabolism in several cell types. As such, they provide further fundamental information to support the so-called fuel concept for the mechanism of glucose-stimulated insulin secretion *(17)*. It is indeed the discovery of the anomeric specificity of both D-glucose metabolism and insulinotropic action in isolated pancreatic islets that yielded the decisive argument in favor of such a fuel concept *(10)*.

Second, these processes should be considered in the framework of the phenomenon of so-called B-cell glucotoxicity or incompetence. Thus, a perturbation of the anomeric specificity of glucose-stimulated insulin release, whether in subjects affected by type-2 diabetes mellitus *(18)* or in animal models for this disease *(19–22)*, represents one of the two major functional aspects of such a phenomenon. It was defined as an anomeric malaise and found attributable to the accumulation of large amounts of glycogen in insulin-producing B-cells in situations of sustained hyperglycemia *(23)*. Incidentally, the proposed enzyme-to-enzyme channeling of α-D-glucose 6-phosphate to phosphoglucoisomerase is perfectly compatible with the latter view. Indeed, we have shown by different procedures that α-D-glucose 6-

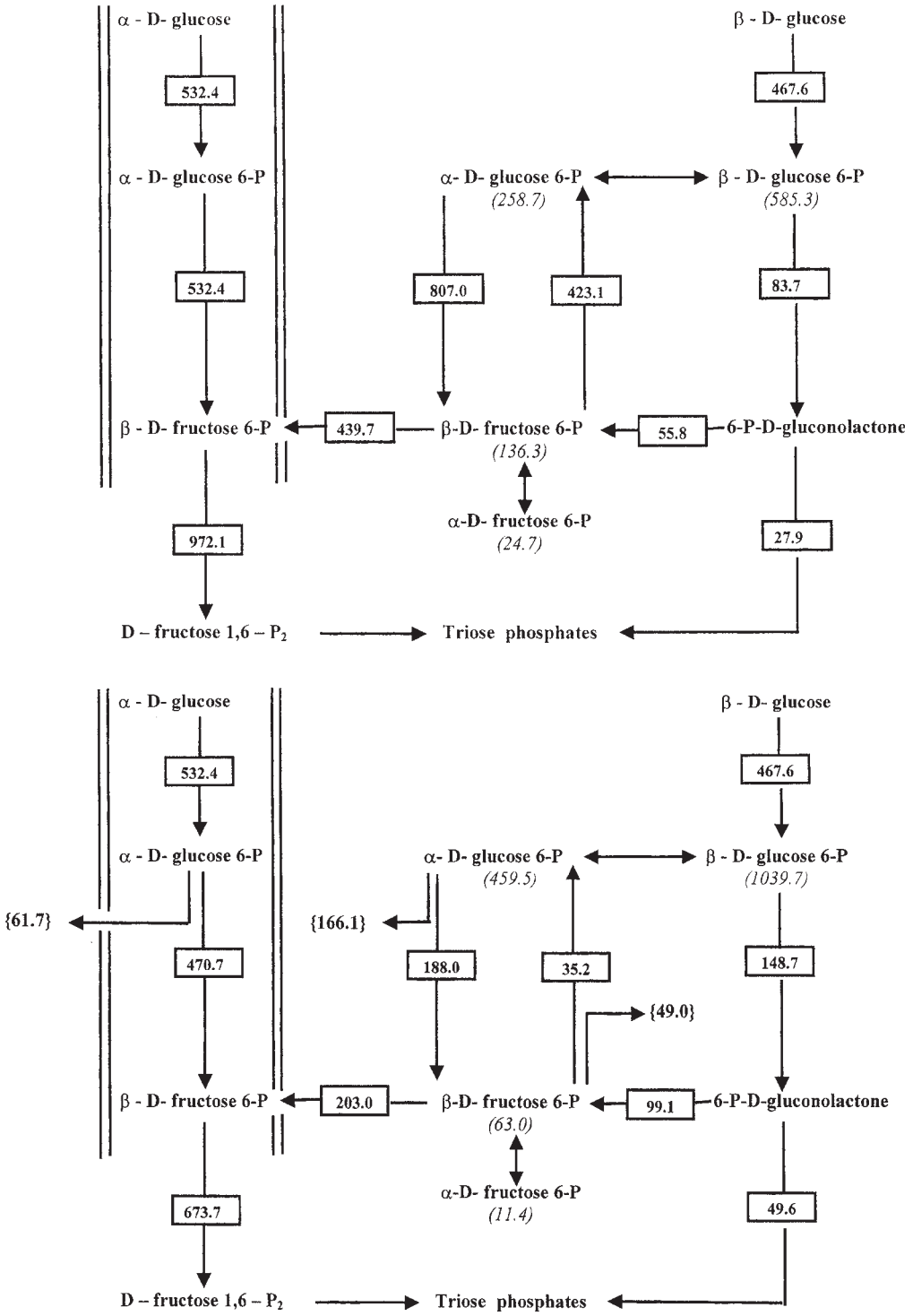

Fig. 3. Schematic view for the metabolism of D-[5-^{3}H]glucose (upper panel) and D-[2-^{3}H]glucose (lower panel) in islets exposed to D-glucose (8.3 m*M*) at anomeric equilibrium. The vertical double lines indicate the enzyme-to-enzyme channeling of α-D-glucose 6-phosphate from its site of formation to phosphoglucoisomerase. Metabolic fluxes are expressed as fmol/min per islet (rectangles and braces) and intracellular contents as fmol per islet (parentheses), the specific radioactivity of the precursor D-glucose molecules being taken as unity. The generation of ^{3}HOH from D-[2-^{3}H]glucose at the level of either bound or free phosphoglucoisomerase is indicated by the braces. In the lower panel, the generation of tritiated triose phosphates from 6-phospho-D-gluconolactone refers to the production of [1-^{3}H]glycerone 3-phosphate from those molecules of D-fructose 6-phosphate emerging from the pentose phosphate pathway and tritiated on their C_3 carbon atom.

phosphate generated by either glycogenolysis or from exogenous D-galactose may undergo enzyme-to-enzyme channeling between phosphoglucomutase and phosphoglucoisomerase *(24,25)*.

Last, it is now obvious that enzyme-to-enzyme channeling in the early steps of glycolysis cannot be ignored if one wishes to reach a sound understanding of ^{3}HOH generation from D-[2-^{3}H]glucose.

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