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Immediate and Delayed Effects of D-Fructose Upon Insulin, Somatostatin, and Glucagon Release by the Perfused Rat Pancreas

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Novel information was recently provided concerning the reciprocal effects of D-glucose and D-fructose upon their respective metabolism in rat pancreatic islets. In the light of such findings, this study aims at comparing the effects of D-glucose and D-fructose on insulin, somatostatin, and glucagon release from the isolated perfused rat pancreas. A rise in D-glucose concentration from 3.3 to 5.0 or 7.3 m*M* or the administration of D-fructose (17 and 40 m*M*) in the presence of 3.3 m*M* D-glucose stimulated insulin release in a concentration-related manner, but failed to affect somatostatin output. The secretion of glucagon was decreased in all cases. The secretory response to L-arginine (5 m*M*), 25 min after restoring the basal concentration of D-glucose, was more markedly affected, in terms of potentiation of insulin and somatostatin release and reduction of glucagon output, after prior administration of D-fructose than after a prior increase in D-glucose concentration. These findings argue against any major role for a paracrine regulation of hormonal release and, instead, are consistent with a causal link between metabolic and secretory events in the islet cells. Nevertheless, the present results emphasize differences in the response of distinct pancreatic endocrine cell types to the same or distinct hexoses.

Key Words: D-glucose; D-fructose; isolated perfused rat pancreas; insulin secretion; somatostatin secretion; glucagon secretion.

Introduction

Although first considered as devoid of any significant insulin-releasing action *(1,2)*, D-fructose was later found to stimulate insulin release, e.g., from rat pancreatic islets *(3,4)*. The possible cause-to-effect link between the metabolism of the ketohexose in islet cells and its insulinotropic action was also investigated *(4–6)*. For instance, it was speculated that a low level of the fructose transporter GLUT5 in insulin-producing B-cells may account for the poor insulinotropic efficiency of D-fructose *(7)*. Yet, rapid equilibration of the ketohexose across the plasma membrane by islet cells had been previously documented *(8)*. More recent findings on the reciprocal effects of D-glucose and D-fructose upon their respective metabolism in islet cells *(9–12)* have renewed interest on this issue. For instance, D-glucose was found to confer, with anomeric specificity, positive cooperativity of glucokinase toward D-fructose *(13,14)*. The ionic, functional, and physiological relevance of such metabolic interactions were also documented *(14–16)*.

In the light of these recent contributions, the major aim of the present study was to compare the effects of D-glucose and D-fructose upon insulin, somatostatin, and glucagon release by the isolated perfused rat pancreas. The delayed effects of these two hexoses upon the later hormonal response to L-arginine was also investigated. To our knowledge, only scanty information was so far available on the effects of D-fructose upon somatostatin and glucagon release. In prior studies conducted in either isolated Syrian hamster islets or the isolated perfused dog pancreas, D-fructose (5.6–16.7 m*M*) was reported not to affect either somatostatin or glucagon secretion *(17,18)*. Sumida et al. claimed that D-fructose (3.3 and 20.0 m*M*) inhibits glucagon output from the isolated rat pancreas *(19)*. In the same study, however, 2-deoxy-D-glucose was also reported to inhibit glucagon secretion, at variance with the observations made by Leclercq-Meyer et al. *(20)*.

Results

Basal Insulin, Somatostatin, and Glucagon Release (min 20–33)

The secretory rates of insulin, somatostatin, and glucagon were comparable in the four series of experiments, during the basal period in the presence of D-glucose (3.3 m*M*). As expected, the insulin and somatostatin outputs were low, with mean values of 0.1 ± 0.1 ng/min and 3.2 ± 0.4 pg/min, respectively (n = 16). The output of glucagon amounted to a mean value of 187 ± 29 pg/min (n = 16).

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Table 1
Insulin, Somatostatin, and Glucagon Release from the Perfused Rat Pancreas, at Selected Times of Experiments Conducted with D-glucose (1.7 or 4 m*M*) and D-fructose (17 or 40 m*M*)[a]

		D-glucose 1.7 m*M*	D-fructose 17 m*M*	D-glucose 4 m*M*	D-fructose 40 m*M*
Insulin release (ng/min)					
Basal period	(min 20–33)	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
Stimulatory period	(min 37–50)	0.1 ± 0.1	0.1 ± 0.1	0.3 ± 0.1	0.8 ± 0.3
Post-stimulatory period	(min 50–60)	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
L-arginine stimulus	(min 73–90)	0.2 ± 0.1	0.5 ± 0.1	0.3 ± 0.1	0.3 ± 0.1
Somatostatin release (pg/min)					
Basal period	(min 20–33)	3.3 ± 0.8	3.2 ± 0.5	2.0 ± 0.5	4.5 ± 1.2
Stimulatory period	(min 37–50)	1.8 ± 0.4	2.5 ± 0.3	1.7 ± 0.3	4.0 ± 1.0
Post-stimulatory period	(min 50–60)	1.9 ± 0.3	2.8 ± 0.1	1.9 ± 0.3	3.1 ± 0.7
L-arginine stimulus	(min 73–90)	2.6 ± 0.4	6.2 ± 2.7	1.0 ± 0.1	6.5 ± 2.9
Glucagon release (pg/min)					
Basal period	(min 20–33)	212 ± 65	234 ± 98	131 ± 37	168 ± 13
Stimulatory period	(min 37–50)	96 ± 16	84 ± 12	60 ± 15	54 ± 5
Post-stimulatory period	(min 50–60)	109 ± 18	89 ± 11	71 ± 13	65 ± 6
L-arginine stimulus	(min 73–90)	1586 ± 127	1030 ± 67	1382 ± 333	732 ± 200

[a]The basal medium contained a low concentration of D-glucose (3.3 m*M*). A L-arginine stimulus (5.0 m*M*) was given at the end of the experiments. Values are means ± SE ($n = 4$ in each series of experiments).

*Comparison of the Effects of 1.7 m*M *D-glucose and 17 m*M *D-fructose upon Insulin, Somatostatin, and Glucagon Release*

The administration of 1.7 m*M* D-glucose only marginally influenced the low basal secretion of insulin seen at 3.3 m*M* D-glucose (Fig. 1, upper panel; Fig. 5, upper left panel; Table 1, first column). The slight and short-lived increase in insulin output, which became apparent using the extended scale (Fig. 5, upper left panel), resulted from only one out of four experiments, in which the secretory rates doubled from a prestimulatory value of 0.2 ng/min (min 33) to that of 0.4 ng/min (min 36). The 10 times higher concentration of 17 m*M* D-fructose did not modify the basal secretory rates of insulin (Fig. 2, upper panel; Fig. 5, upper right panel; Table 1, second column).

The basal release of somatostatin was not significantly influenced by the administration of 1.7 m*M* glucose (Fig. 1, middle panel; Table 1, first column). In response to 17 m*M* D-fructose, a rather late and short-lived increase in output occurred (Fig. 2, middle panel, min 40, 4.6 ± 2.1 pg/min). As a whole, however, the output of somatostatin during the 17 m*M* D-fructose stimulatory period was not significantly different from that seen during the basal prestimulatory period (Table 1, second column).

Despite their limited effects on insulin and somatostatin release, both 1.7 m*M* D-glucose and 17 m*M* D-fructose inhibited the secretion of glucagon (Figs. 1 and 2, lower panel; Fig. 6, upper panels). Thus, the output of glucagon was reduced from a basal value of 212 ± 65 to 96 ± 16 pg/min upon the administration of 1.7 m*M* D-glucose, and from 234 ± 98 to 84 ± 12 pg/min upon the administration of 17 m*M* D-fructose (Table 1, first and second columns). In terms of percentages, the observed inhibitions were comparable and represented mean values of 50 ± 6% and 51 ± 12% in response to 1.7 m*M* D-glucose and 17 m*M* D-fructose, respectively. The inhibition of glucagon release was borne out by the finding that the release of glucagon was significantly higher (+10.8 ± 4.5%; $n = 8$; $p < 0.05$) after (min 50–60) than during (min 37–50) administration of the two hexoses.

*Comparison of the Effects of 4 m*M *D-glucose and 40 m*M *D-fructose upon Insulin, Somatostatin and Glucagon Release*

These higher concentrations of D-glucose and D-fructose induced notable and biphasic insulin secretory responses from the perfused pancreas (Figs. 3 and 4, upper panels; Fig. 5, lower panels). Both the early and late phases of stimulated insulin release were slightly higher in response to 40 m*M* D-fructose than 4 m*M* D-glucose. Such a difference failed, however, to achieve statistical significance (Table 1, third and fourth column, min 37–50, 0.8 ± 0.3 in comparison to 0.3 ± 0.1 ng/min, $p = 0.2$). Both the 4 m*M* D-glucose and 40 m*M* D-fructose stimulations of insulin release were readily reversible (Table 1, third and fourth columns, post-stimulatory periods, min 50–60, 0.1 ± 0.1 ng/min).

In contrast to insulin release, neither 4 m*M* D-glucose nor 40 m*M* D-fructose stimulated the release of somatostatin (Figs. 3 and 4, middle panels). Thus, the output of somatostatin was comparable during the basal and stimulatory periods (Table 1, third and fourth columns).

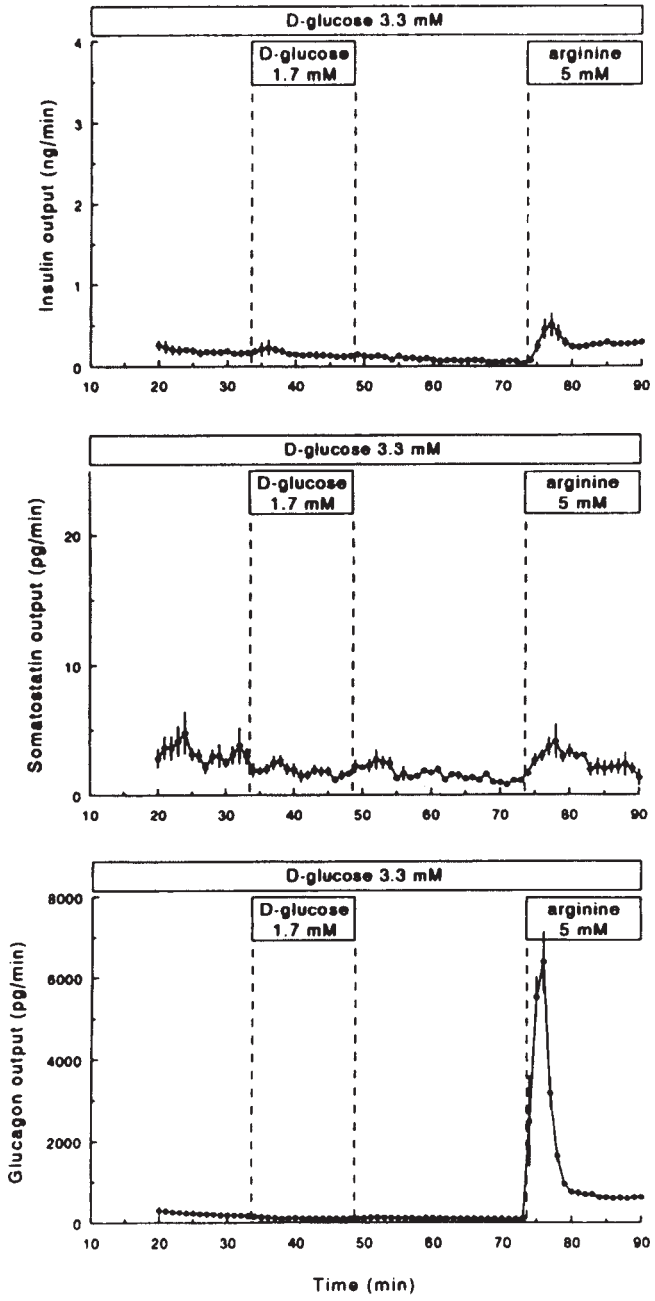

Fig. 1. Time course for changes in insulin (upper panel), somatostatin (middle panel), and glucagon (lower panel) output at 3.3 m*M* D-glucose, in pancreases exposed to an 1.7 m*M* increment in D-glucose concentration (min 33–48) and 5 m*M* L-arginine (min 73–90). The results refer to mean values ± SE ($n = 4$).

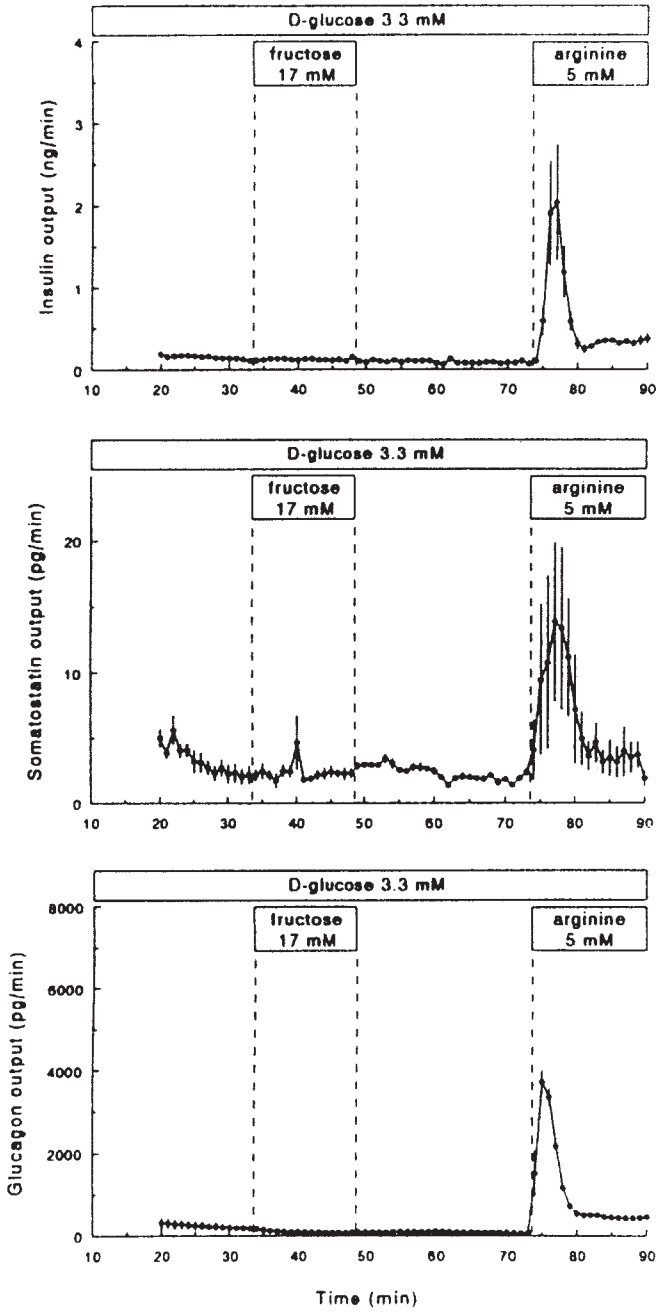

Fig. 2. Time course for changes in insulin (upper panel), somatostatin (middle panel), and glucagon (lower panel) output at 3.3 m*M* D-glucose, in pancreases exposed to 17 m*M* D-fructose (min 33–48) and 5 m*M* L-arginine (min 73–90). The results refer to mean values ± SE ($n = 4$).

As already observed at low concentrations, the higher concentrations of D-glucose and D-fructose inhibited the secretion of glucagon (Figs. 3 and 4, lower panels; Fig. 6, lower panels). Thus, the output of glucagon was reduced from a basal value of 131 ± 37 to 60 ± 15 pg/min upon the administration of 4 m*M* D-glucose, and from 168 ± 13 to 54 ± 5 pg/min upon the administration of 40 m*M* D-fructose (Table 1, third and fourth columns, min 20–33 in comparison to min 37–50). In terms of percentages, the observed inhibitions were statistically comparable and represented mean values of 46 ± 12% and 67 ± 4% in the case of 4 m*M* D-glucose and 40 m*M* D-fructose, respectively. Once again, the inhibition of glucagon output was borne out by the fact that it was significantly higher (+23.0 ± 8.8%; $n = 8$; $p < 0.05$) after (min 50–60) than during (min 37–50) administration of the hexoses (Fig. 6, lower panels).

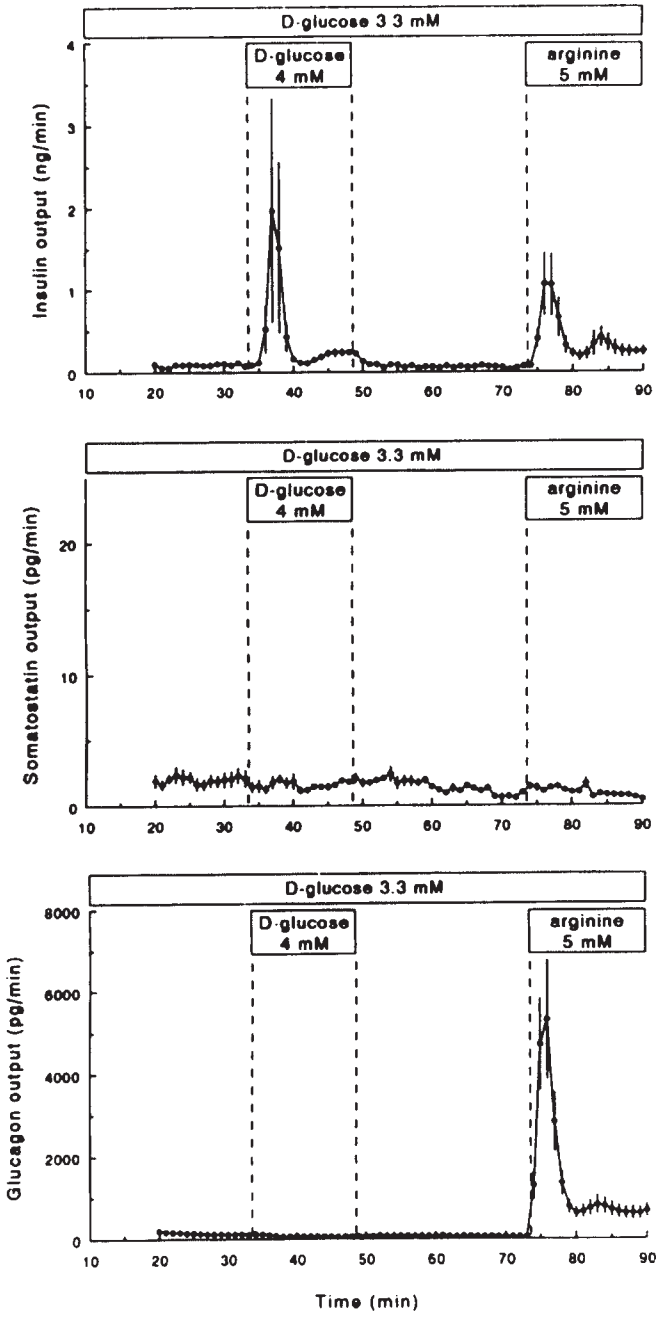

Fig. 3. Time course for changes in insulin (upper panel), somatostatin (middle panel), and glucagon (lower panel) output at 3.3 m*M* D-glucose, in pancreases exposed to an 4 m*M* increment in D-glucose concentration (min 33–48) and 5 m*M* L-arginine (min 73–90). The results refer to mean values ± SE ($n = 4$).

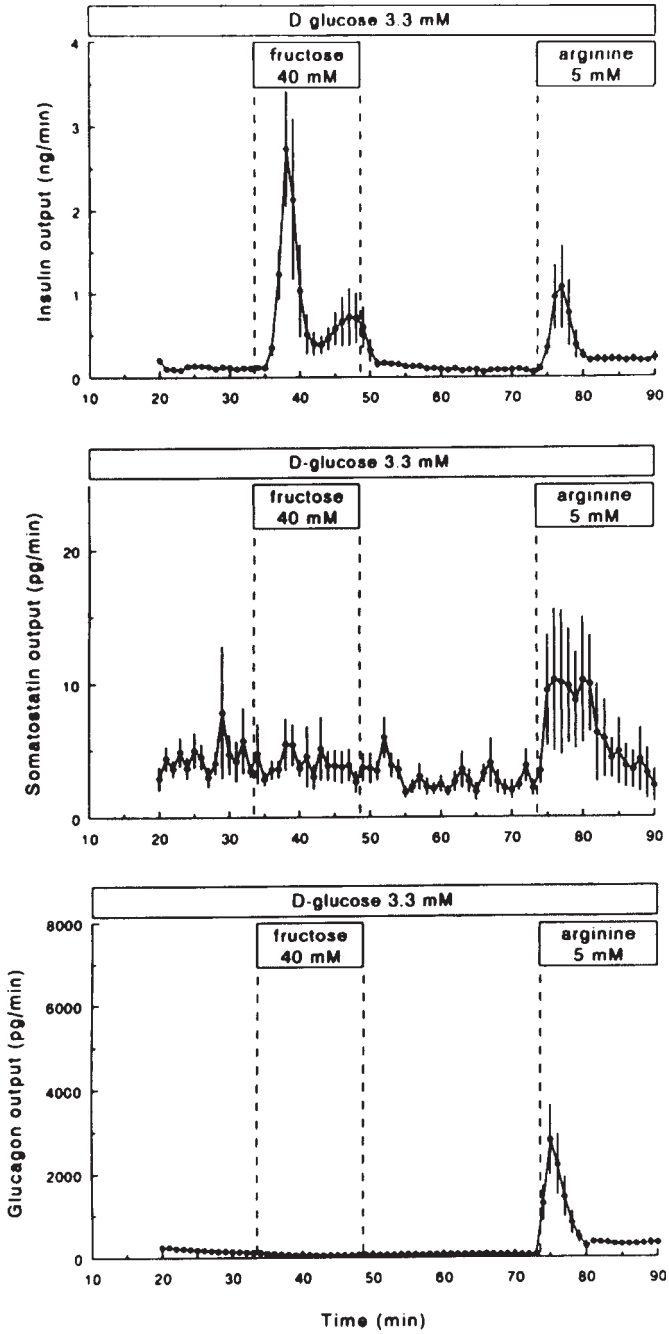

Fig. 4. Time course for changes in insulin (upper panel), somatostatin (middle panel), and glucagon (lower panel) output at 3.3 m*M* D-glucose, in pancreases exposed to 40 m*M* D-fructose (min 33–48) and 5 m*M* L-arginine (min 73–90). The results refer to mean values ± SE ($n = 4$).

*Effects of the Late 5 m*M *L-arginine Stimulus upon Insulin, Somatostatin, and Glucagon Release (min 73–90)*

In the presence of 3.3 m*M* D-glucose, and after earlier exposure to 1.7 m*M* D-glucose, L-arginine induced a biphasic increase in insulin release (Fig. 1, upper panel; Table 1, first column). The insulin response to L-arginine was slightly, but not significantly, amplified after prior exposure to 4 m*M* D-glucose and 40 m*M* D-fructose (Figs. 3 and 4, upper panels, Table 1, third and fourth columns). The highest amplification ($p < 0.05$) was observed after prior exposure to 17 m*M* D-fructose (Fig. 2, upper panel; Table 1, second column). The same conclusions were reached when the response to L-arginine was judged from the paired increase in insulin output before (min 50–60) and during (min 73–90) administration of the amino acid (data not shown).

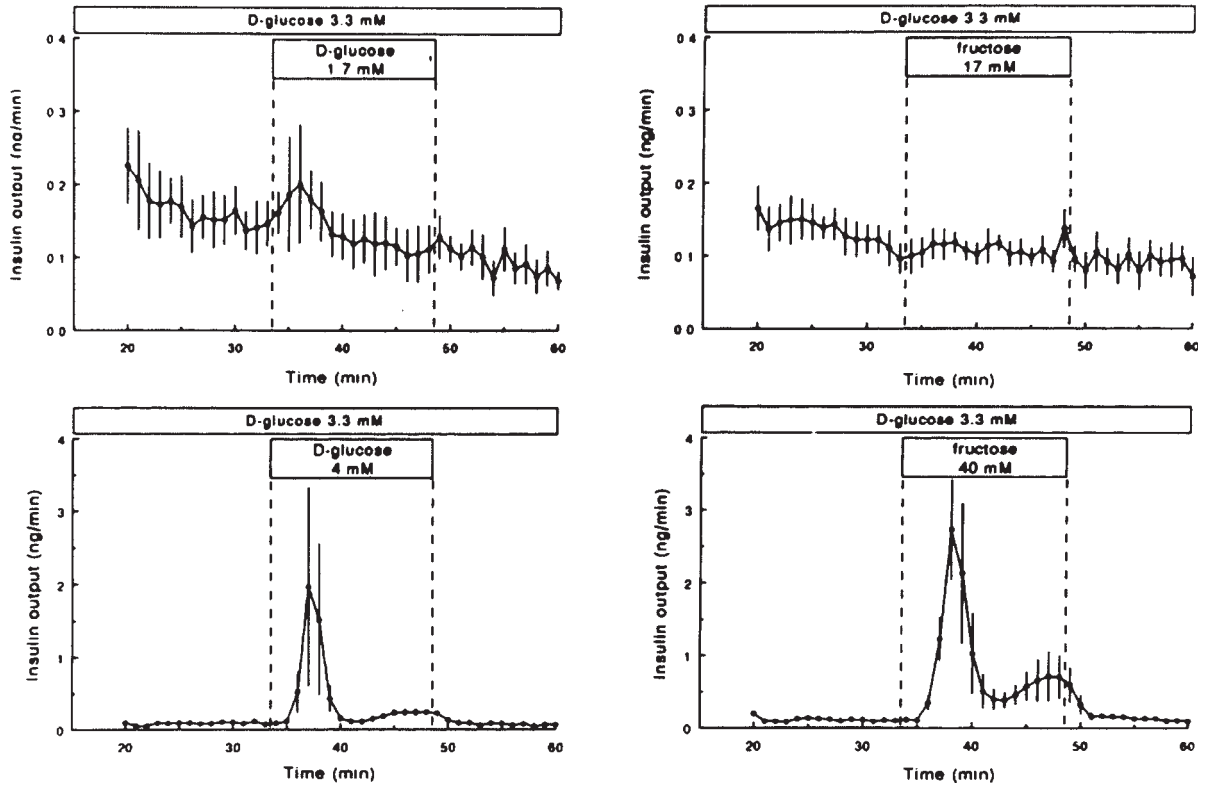

Fig. 5. Insulin release in the same experiments as those illustrated in the upper panels of Figs. 1 to 4, but here drawn with an extended ordinate scale for the first 60 min of perfusion. The results obtained, at 3.3 m*M* D-glucose, in response to an 1.7 and 4 m*M* increment D-glucose concentration (left upper and lower panels), and to 17 and 40 m*M* D-fructose (right upper and lower panels) refer to four individual experiments in each case.

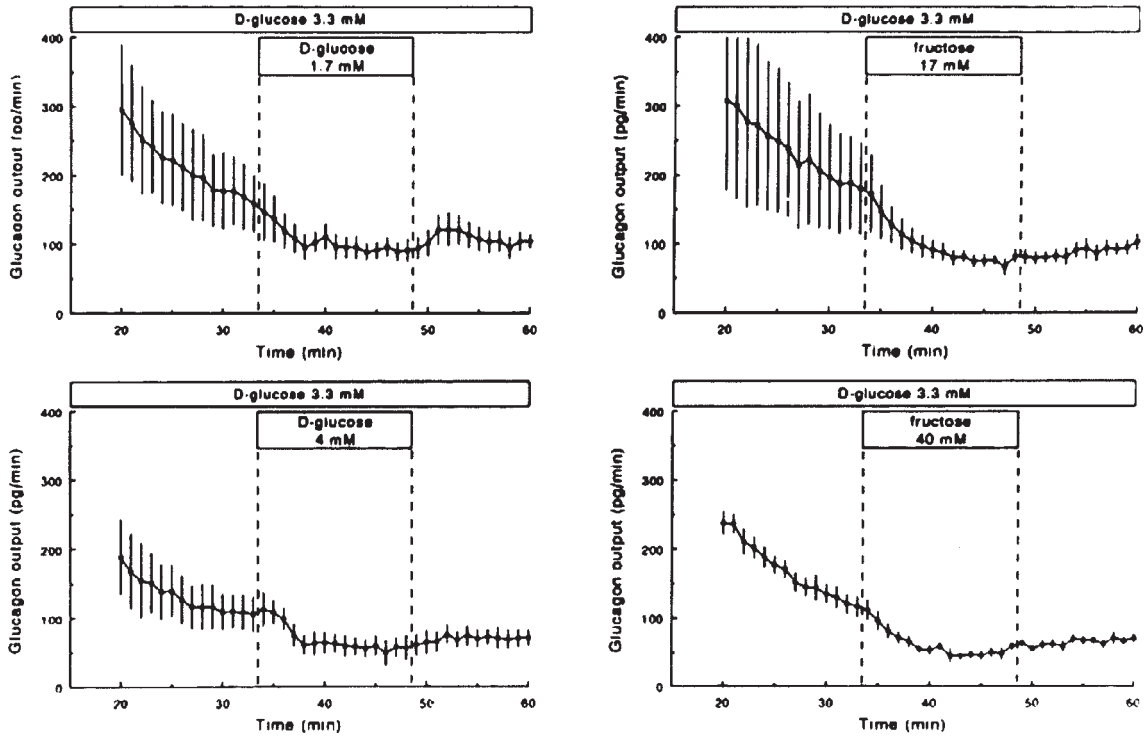

Fig. 6. Glucagon release in the same experiments as those illustrated in the lower panels of Figs. 1 to 4, but here drawn with an extended ordinate scale for the first 60 min of perfusion. The results obtained, at 3.3 m*M* D-glucose, in response to an 1.7 and 4 m*M* increment in D-glucose concentration (left upper and lower panels), and to 17 and 40 m*M* D-fructose (right upper and lower panels) refer to four individual experiments in each case.

The somatostatin response to L-arginine was quite limited and often even absent after prior exposure to 1.7 or 4 m*M* D-glucose (Figs. 1 and 3, middle panels). After prior exposure to either 17 or 40 m*M* D-fructose, however, a sizable somatostatin response to L-arginine was usually observed (Figs. 2 and 4, middle panels). The release of somatostatin during exposure to L-arginine (min 73–90) averaged 1.8 ± 0.3 pg/min ($n = 8$) after prior exposure to 1.7 and 4.0 m*M* D-glucose, as distinct ($p < 0.05$) from 6.4 ± 1.8 pg/min ($n = 8$) after prior exposure to 17 and 40 m*M* D-fructose. As a matter of fact, the paired changes in somatostatin output before (min 50–60) and during (min 73–90) L-arginine administration failed to achieve statistical significance (–0.1 ± 0.4 pg/min; $n = 8$; $p > 0.7$) after prior exposure to D-glucose (1.7 and 4.0 m*M*), while averaging +3.4 ± 1.8 pg/min ($n = 8$) after prior exposure to D-fructose (17 and 40 m*M*). Even the paired difference in somatostatin output between the highest value recorded during exposure to L-arginine (min 78.4 ± 0.9; $n = 16$) and that recorded at min 70 averaged no more than 2.7 ± 0.7 pg/min ($n = 8$) after prior exposure to D-glucose (1.7 or 4.0 m*M*), as distinct ($p < 0.05$) from 11.8 ± 4.0 pg/min ($n = 8$) after prior exposure to D-fructose (17 or 40 m*M*).

The glucagon secretory response to L-arginine amounted to 1586 ± 127 pg/min after prior exposure to the lowest concentration of 1.7 m*M* D-glucose (Fig. 1, lower panel; Table 1, first column). Such a L-arginine–induced glucagon release was slightly, although not significantly, reduced to a value of 1382 ± 333 pg/min after prior exposure to 4 m*M* D-glucose (Fig. 2, lower panel; Table 1, third column). Prior exposure to either 17 m*M* or 40 m*M* D-fructose, however, was followed by a highly significant and dose-related reduction of the glucagon response to L-arginine (Figs. 2 and 3, lower panels; Table 1, second and fourth column, 1030 ± 67 and 732 ± 200 pg/min; $p < 0.01$ and 0.02, respectively).

Perfusion Pressure

The administration of 1.7 or 4 m*M* D-glucose, as well as 17 m*M* D-fructose or 5 m*M* L-arginine, did not modify the perfusion pressure recorded in the experiments (Fig. 7). In response to 40 m*M* D-fructose, a slight, but nevertheless significant decrease in perfusion pressure was observed (Fig. 7, lower panel, min 57–60, 23 ± 3 mmHg, relative to 24 ± 3 mmHg during the min 20–33 prestimulatory period, paired analysis, $p < 0.05$).

Discussion

The major findings emerging from the present study can be summarized as follows.

First, in the case of insulin, a rise in D-glucose concentration from 3.3 to 5.0 m*M* or the administration of 17 m*M* D-fructose in the presence of 3.3 m*M* D-glucose failed, as a rule, to cause any sizable increase in hormonal release. However, a rise in D-glucose concentration from 3.3 to 7.3 m*M*

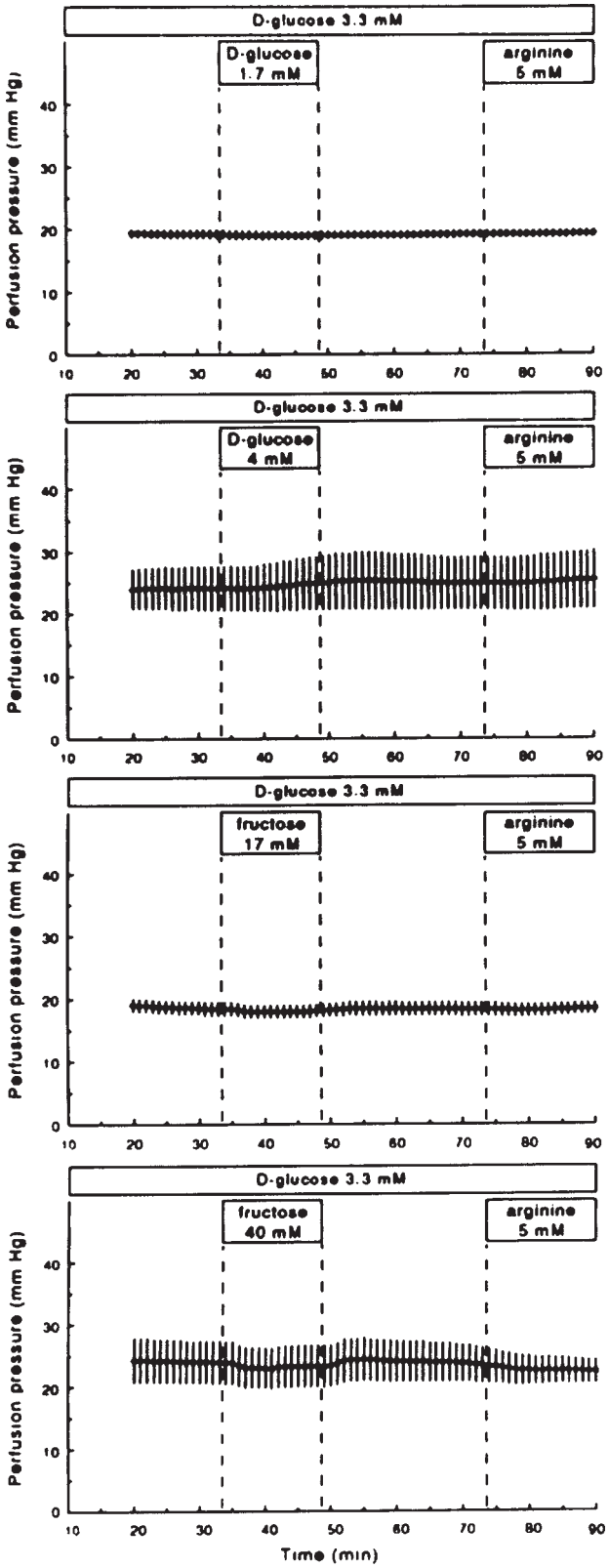

Fig. 7. Perfusion pressure in the isolated pancreas perfused at 3.3 m*M* D-glucose and exposed, first between min 33 to 48, to a 1.7 m*M* (upper panel) and 4.0 m*M* (second panel) increment in D-glucose concentration or to 17 m*M* (third panel) and 40 m*M* (lower panel) D-fructose, and later (min 73–90) to 5 m*M* L-arginine. Mean values (± SE) refer to four individual experiments in each case.

or the administration of 40 m*M* D-fructose in the presence of 3.3 m*M* D-glucose caused marked, biphasic, and rapidly reversible stimulation of insulin release, the magnitude of such a secretory response being comparable in both cases. When L-arginine (5 m*M*) was administered 25 min after restoring the hexose concentration to only 3.3 m*M* D-glucose, the output of insulin was significantly higher after prior administration of 17 m*M* D-fructose than after prior exposure to an 1.7 m*M* increment in D-glucose concentration. In this respect, the arginine-induced increment in insulin output found after prior exposure to either a 4.0 m*M* increment in D-glucose concentration (333 ± 105 pg/min) or 40 m*M* D-fructose (297 ± 103 pg/min) yielded mean values in between those observed after exposure to an 1.7 m*M* increment in D-glucose concentration (245 ± 30 pg/min) and 17 m*M* D-fructose (509 ± 102 pg/min). These findings are consistent with a threshold concentration for the insulinotropic action of D-glucose close to 5.0 m*M* and with a concentration-related secretory response to D-fructose in the 17 to 40 m*M* range *(1,5)*. The memory toward the hexoses, in terms of modulation of the later response to L-arginine, was somewhat higher in the case of D-fructose than D-glucose, with overall respective mean values for the arginine-induced increment in insulin output of 289 ± 53 and 358 ± 80 pg/min ($n = 6$ in both cases; $p > 0.5$).

Second, in the case of somatostatin, neither an increase in D-glucose concentration from 3.3 to 5.0 or 7.3 m*M* nor the administration of D-fructose (17 or 40 m*M*) in the presence of 3.3 m*M* D-glucose provoked any obvious change in secretory rate. Nevertheless, the later secretory response to L-arginine was higher after prior administration of D-fructose than after a prior rise in D-glucose concentration, in which case it even failed to achieve statistical significance when computed over the entire period of exposure to the cationic amino acid. These data reveal that D-fructose is more efficient than D-glucose, tested at concentrations of close-to-equal insulinotropic potencies, in causing enhancement of the later secretory response to L-arginine.

Last, in the case of glucagon, a rapid, sustained, and rapidly reversible decrease in hormonal output was always observed in response to either a rise in D-glucose concentration or the administration of D-fructose. The later administration of L-arginine augmented glucagon output. Such a secretory effect was highest after prior exposure to a rise in D-glucose concentration from 3.3 to 5.0 m*M*, somewhat lower after a prior increment in D-glucose concentration from 3.3 to 7.3 m*M*, and further decreased, in a concentration-related manner, after prior administration of D-fructose (17 and 40 m*M*). These data confirm the exquisite sensitivity of the glucagon-producing cells to a rise in hexose concentration *(18, 21)*. They further document, as already observed in the case of insulin and somatostatin output, a higher efficiency of D-fructose than D-glucose in terms of modulation of the later secretory response to L-arginine.

The results recorded for insulin and glucagon release in response to D-glucose and D-fructose argue against any major role of a paracrine effect in the regulation of either glucagon secretion by insulin or insulin output by glucagon. Thus, on the one hand, the hexoses inhibited glucagon release under conditions in which they failed to stimulate insulin secretion. On the other hand, the hexoses enhanced insulin output at a time when they inhibited glucagon secretion. Instead, the present data are consistent with the existence of a link between the metabolic and secretory responses of insulin- and glucagon-producing cells to D-glucose and D-fructose. For instance, the insulinotropic action of the hexoses paralleled their capacity to undergo catabolism in isolated rat pancreatic islets *(3,22)*. This is not meant to deny that the metabolic response of insulin- and glucagon-producing cells may differ from one another, as suggested by the higher sensitivity of the latter than former cells to low concentrations of D-glucose or D-fructose. Such a difference is consistent with the view that, in glucagon-producing cells, but not so in insulin-producing cells, the transport of hexoses across the plasma membrane may represent a rate-limiting step in their further catabolism *(23)*.

It seems established from prior observations that the stimulation of somatostatin release by D-glucose is also causally linked to the metabolism of the hexose in islet cells. Such a stimulation indeed displays anomeric preference for α- vs β-D-glucose *(24)*, and is suppressed by D-mannoheptulose *(25)* or 2-deoxy-D-glucose *(20)*. Once again, however, the metabolism of hexoses may differ in the somatostatin-producing cells and other endocrine pancreatic cells. This is suggested by the finding that a rise in D-glucose concentration, even up to 7.3 m*M*, or the administration of D-fructose, even at a 40 m*M* concentration, failed to stimulate somatostatin release, in sharp contrast to the results recorded in the case of insulin secretion.

The results concerning the modulation of the islet cell secretory response to L-arginine by the prior exposure to a rise in D-glucose concentration or the administration of D-fructose may require a more nuanced interpretation. As a rule, the prior administration of D-fructose was more efficient than a prior rise in D-glucose concentration in modulating the secretory response of the islet cells to the later exposure to L-arginine. Such a difference was most obvious in the case of somatostatin- and glucagon-producing cells. The interpretation of these unexpected findings is open to speculation. For instance, the relative metabolic efficiency of the two hexoses could differ in distinct types of pancreatic endocrine cells. Alternatively, it could be postulated, as already proposed elsewhere *(6,26)*, that the effect of D-fructose upon functional variables in endocrine pancreatic cells is not solely accounted for by the catabolism of the ketohexose in these cells.

In view of the high concentrations of D-fructose used in the present experiments, the relevance of the present findings

Table 2
Characteristics of Rats
and Experimental Variables of the Perfusions

Rat weight (g)	381 ± 22 (16)
Plasma glucose (mmol/L)	3.6 ± 0.2 (16)
Plasma insulin (μg/L)	0.84 ± 0.16 (16)
Pancreas wet weight (g)	1.162 ± 0.055 (16)
Pancreas insulin content	
μg	152 ± 9 (16)
μg/g	133 ± 8 (16)
Pancreas somatostatin content	
μg	0.48 ± 0.04 (16)
μg/g	0.42 ± 0.03 (16)
Pancreas glucagon content	
μg	6.2 ± 0.4 (16)
μg/g	5.5 ± 0.3 (16)
Insulin/somatostatin ratio (molar)	95 ± 8 (16)
Insulin/glucagon ratio (molar)	14.6 ± 0.9 (16)
Glucagon/somatostatin (molar)	6.7 ± 0.7 (16)
Flow rate (mL/min)	1.51 ± 0.02 (16)
Perfusion pressure (mmHg)	
min 20	21.6 ± 1.4 (16)
min 90	21.3 ± 1.4 (16)

to the physiology of insulin secretion in vivo could be questioned. Nevertheless, it should be stressed that stimulation of insulin release by D-fructose in vivo was recently documented in response to oral administration of either the ketohexose or sucrose to normal rats *(16)*.

In conclusion, therefore, the present results emphasize the view that the secretory response of distinct hormonal cell types in the endocrine pancreas to distinct hexoses does not always display an identical pattern.

Materials and Methods

Male Wistar rats (Iffa Credo, L'Arbresle, France) were used in the present experiments ($n = 16$). They were fasted overnight, and their body weight averaged 381 ± 22 g (Table 2). The glucose and insulin concentrations, in a plasma sample obtained from the tail prior to anesthesia, amounted to 3.6 ± 0.2 m*M* and 0.84 ± 0.16 μg/L, respectively. The animals were anesthetized with pentobarbital sodium (0.2 mmol/kg, ip), and the pancreas was perfused without recirculation through both the celiac and superior mesenteric arteries, as previously described *(20,27)*. This protocol was approved by the Commission d'Ethique et du Bien-Etre Animal of our Faculty.

The basal salt-balanced solution contained dextran (clinical grade; 40 g/L; Sigma), bovine serum albumin (RIA grade; 5 g/L; Sigma), and D-glucose (3.3 m*M*). The D-glucose (1.7 or 4.0 m*M*), D-fructose (17 or 40 m*M*), and L-arginine (5 m*M*) stimuli were dissolved in the basal perfusion medium using separate reservoirs. The final concentrations of D-glucose in the perfusate during the D-glucose stimulatory periods were thus 4.0 or 7.3 m*M*. These concentrations of the hexoses were selected because comparable rates of insulin release are observed in rat pancreatic islets exposed to about 4.0 m*M* D-glucose or both 3.3 m*M* D-glucose and 17 m*M* D-fructose, and 7.3 m*M* D-glucose or both 3.3 m*M* D-glucose and 40 m*M* D-fructose *(4)*. All solutions were continuously gassed (95% O_2:5% CO_2; pH 7.4) and directed to the pancreas–duodenum preparation at a temperature of 37°C with a peristaltic pump (Minipuls 3, Gilson, Villiers-le-Bel, France). The overall mean value averaged 1.51 ± 0.02 mL/min for the flow rate, and 21.6 ± 1.4 and 21.3 ± 1.4 mmHg for the perfusion pressure at min 20 and 90, respectively. The pancreas wet weight at the end of the perfusion averaged 1.162 ± 0.055 g. Its insulin content amounted to 152 ± 9 μg (133 ± 9 μg/g), that of somatostatin 0.48 ± 0.04 μg (0.42 ± 0.03 μg/g), and that of glucagon 6.2 ± 0 4 μg (5.5 ± 0.3 μg/g). The resulting molar ratios represented 95 ± 8 (insulin/somatostatin), 14.6 ± 0.9 (insulin/glucagon), and 6.7 ± 0.7 (glucagon/somatostatin), respectively.

The techniques used for the measurement of plasma glucose and insulin concentrations, perfusion pressure, pancreatic insulin, somatostatin and glucagon content and release were identical to those described previously *(28,29)*.

The mean hormonal outputs recorded in the four series of experiments are illustrated using a comparable scale in Figs. 1 to 4. The results obtained for insulin and glucagon release during the early parts of the perfusions (min 20–60) were also drawn using an enhanced scale in order to document, at an appropriate magnification, the changes in secretory rates that occurred prior to the late administration of L-arginine (Figs. 5 and 6). The vertical dotted lines on the graphs were corrected for the dead space of the perfusion device *(21)*.

The mean hormonal output in each individual experiment over a given period of time was computed by planimetry from all measurements made over that period.

All results, including those already mentioned, are presented as means ± SE together with the number of individual observations (n). The statistical significance of differences between mean values was assessed by use of Student's two-tailed paired or unpaired t-test or one way analysis of variance (ANOVA) as appropriate (Instat 2, Graphpad Software, San Diego, CA, USA). The null hypothesis was rejected for p values < 0.05.

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