Hypersensitivity to arginine of both B and D pancreatic cells in adult streptozotocin-diabetic rats

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Abstract. Insulin and pancreatic somatostatin secretions were studied after stimulation with an arginine infusion (5 mmol/l) in isolated perfused pancreata of adult streptozotocin-diabetic rats. In the presence of 2.8 mmol/l glucose, arginine clearly stimulated insulin and somatostatin secretions in diabetic rats, whereas it was ineffective in normal rats. Thus, not only the B-cells, but also the D-cells of the pancreas from streptozotocin-diabetic rats are hypersensitive to arginine. The infusion of insulin (4 U/l) did not modify this hypersensitivity of the D-cells to arginine in pancreata of streptozotocin-diabetic rats.

Since the diabetogenic action of streptozotocin (STZ) has been described (1, 2), pancreatic hormonal responses during STZ-diabetes have been extensively studied. Hypersensitivity of the B-cells to arginine stimulation has been reported in the pancreas of rats made diabetic by streptozotocin on the day of their birth (3) or 2 days after birth (4). On the other hand, arginine is known to stimulate not only insulin, but also pancreatic somatostatin secretion in the normal rat (5–7). Moreover, Hara et al. (8) showed that arginine-induced somatostatin secretion was increased in alloxan diabetic rats. So, in the present work we have investigated whether an hypersensitivity to arginine could be observed in the pancreas of rats rendered diabetic by STZ at the adult state, not only for B-cells but also for D-cells. The study was performed in isolated, perfused rat pancreata. The glucose concentration of the perfusion medium was chosen low (2.8 mmol/l) to prevent stimulation of insulin and somatostatin secretions by arginine, in normal rats.

Our experiments permit us to observe an hypersensitivity to arginine of the D-cells in diabetic rats. As STZ diabetes is characterized by insulin deficiency and as it is known that exogenous insulin can reduce pancreatic somatostatin secretion in vitro (9) and in vivo (10) experiments, we investigated whether D-cell hypersensitivity could be suppressed by a continuous insulin infusion.

Materials and Methods

Animals
We used adult male Wistar rats (Janvier, le Genest 53940, St Berthevin, France) which were housed in individual metabolic cages and had free access to food (UAR, Villemoisson, 91360, Epinay sur Orge, France) and water. After a 10-day acclimation period their weight ranged between 340 and 355 g. Rats were randomly assigned to 2 groups: control normal animals and animals designed to become diabetic; the latter received an injection of streptozotocin (Upjohn Co, Kalamazoo, MI) at the usual dose of 66 mg/kg ip (11). The diabetic state was ensured by daily measurements of glucosuria which ranged from 10 to 12 g/24 h. The pancreas was isolated 3 weeks after induction of diabetes.

Pancreas isolation procedure
Animals fed ad libitum, were anesthetized with sodium pentobarbital (60 mg/kg ip). The pancreas was completely isolated from all neighbouring tissues and im-
mediately perfused with physiological medium, according to the procedure of Loubatières et al. (12); it was then transferred into a plastic chamber maintained at 37.5°C. The perfusion medium, which was not recirculated, was Krebs Ringer bicarbonate buffer containing 2 g/l bovine serum albumin (Fraction V, Sigma Chemical, St. Louis, MO) and glucose at a concentration of 2.8 mmol/l; it had the following ionic composition: NaCl, 108; K$_2$HPO$_4$, 1.19; KCl, 4.74; CaCl$_2$, 2.54; MgSO$_4$.7H$_2$O, 1.19; NaHCO$_3$, 18 mmol/l and was continuously bubbled with a mixture of 95% O$_2$, 5% CO$_2$ so as to maintain the pH between 7.35 and 7.40. A constant perfusion pressure (in the range of 30–40 cm water) was applied to the organ throughout the experiment, selected so as to provide a pancreatic outflow rate of 2.5 ml/min at the beginning of the experiment. The first sample was taken after an equilibration period of 30 min. Two more samples were taken at 40 and 45 min. L-arginine (Merck, Darmstadt, FRG) at a concentration of 5 mmol/l was infused for 30 min from time 45 to 75 min. For each sampling, pancreatic outflow rate was measured and two aliquots were immediately frozen in chilled tubes for insulin and somatostatin determinations. For the somatostatin assay, 500-µl aliquots were placed in tubes containing 50 µl of a mixture of EDTA (32 mmol/l) and aprotinin (10* KIU/l, Zymophren®, Specia).

In these conditions three sets of experiments were performed: arginine was administered to 1. isolated perfused pancreas of 12 normal rats; 2. isolated perfused pancreas of 9 STZ-diabetic rats, and 3. isolated perfused pancreas of 6 STZ-diabetic rats which received insulin (pork soluble insulin, Endopancrine®, Organon, Sérifontaine, France) (4 U/l) directly into the infusion medium from time 0, immediately after pancreas isolation and throughout the experiment.

In all cases intracardiac blood was sampled at the end of dissection just before taking off the pancreas to determine the blood glucose levels of the animals.

Assays

For evaluation of pancreatic hormones the same radioimmunological methods were used for both Krebs-Ringer buffer and blood. Insulin was assayed by the method of Herbert et al. (13). The insulin antibody used was obtained from Miles Laboratories (Paris, France). The intra- and inter-assay coefficient of variation was 9 and 13.5%, respectively. The analytical sensitivity, defined as the concentration of insulin displacing 5% of the initially bound tracer, was 0.1 µg/l. Immunoreactive somatostatin was assayed in non-extracted samples according to the technique described previously (14) using the 80 G antiseraum, a gift from Dr Unger (Health Science Center, Dallas, TX). The intra- and inter-assay coefficient of variation was 10 and 14.4%, respectively; the sensitivity was 10 ng/l. Blood glucose was measured by the potassium ferricyanide method using a Technicon autoanalyser (15).

Statistics analysis

Results are expressed as mean ± SEM. Insulin and somatostatin output rates were calculated by multiplying the hormone concentration (respectively µg/l and ng/l) by the pancreatic outflow rate (ml/min). Kinetics data were submitted to analysis of variance using the multiple comparison test (16). For each experiment we estimated the area under curve (AUC) during the first 15 min of arginine administration. AUC and in vivo parameters were analysed using Student’s t-test.

Results

Characterization of diabetic state (Table 1)

Three weeks after induction of diabetes, the weight of diabetic rats was clearly lower than that of normal rats (p < 0.01). Intracardiac blood samples ensured the diabetic state of animals treated with STZ: elevated glucosuria and hyperglycemia (p < 0.001) accompanied by a fall of plasma insulin levels (p < 0.01), and an increase in plasma somatostatin levels (p < 0.01).

Normal and STZ-diabetic rat pancreas: effects of arginine on hormone secretions

Insulin release (Fig. 1). Basal insulin secretion of normal rats at 2.8 mmol/l glucose was low (0.95 ± 0.21 ng/min at time 45 min) and this secretion was not modified by arginine infusion. The AUC during the first 15 min was 13.2 ± 3.8.

Basal insulin secretion of diabetic rats was lower but not significantly (0.56 ± 0.14 ng/min at time 45 min). Paradoxically, in these pancreata arginine induced an immediate and transient increase in in-

<table>
<thead>
<tr>
<th></th>
<th>Normal rats (12)</th>
<th>Diabetic rats (15)</th>
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</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>415 ± 5</td>
<td>330 ± 4**</td>
</tr>
<tr>
<td>Blood glucose (mmol/l)</td>
<td>8.6 ± 0.4</td>
<td>33.0 ± 0.7***</td>
</tr>
<tr>
<td>Glucosuria (g/24 h)</td>
<td>0</td>
<td>11.6 ± 0.6***</td>
</tr>
<tr>
<td>Plasma insulin (µg/l)</td>
<td>13.5 ± 2.4</td>
<td>2.5 ± 0.3**</td>
</tr>
<tr>
<td>Plasma somatostatin (ng/l)</td>
<td>73 ± 8</td>
<td>146 ± 13**</td>
</tr>
</tbody>
</table>

* p < 0.05; ** p < 0.01; *** p < 0.001.
sulin output. This augmentation was significant (p < 0.01) during the first 8 min. Then insulin secretion returned to starting values. The AUC during the first 15 min was 26.4 ± 5.2 and significantly higher than that observed in normal pancreata (p < 0.05).

Somatostatin release (Fig. 2). Basal somatostatin secretion was higher in diabetic than in normal rat pancreas (38 ± 6 vs 19 ± 3 pg/min at time 45 min) (p < 0.01). Arginine did not modify the somatostatin output in normal rat pancreas. The AUC during the first 15 min was 331 ± 48.

In contrast, in diabetic rats this amino acid provoked an immediate stimulation of somatostatin release which was significant, but transient (p < 0.01 at 2, 3, 4 and 6 min); subsequently the secretion progressively returned to starting values.

Effect of arginine infusion (5 mmol/l) on somatostatin output rate from isolated perfused rat pancreata of normal rats (○), N = 12, and streptozotocin-diabetic rats (●), N = 9, in the presence of 2.8 mmol/l glucose. Values are mean ± SEM.

Glucose 2.8 mmol/l

Arginine 5 mmol/l

Minutes

Fig. 2.

Effect of insulin infusion (4 U/l) started from the beginning of perfusion. This insulin administration did not induce a normalization of pancreatic somatostatin secretion either in basal or arginine stimulated conditions. The AUC during the first 15 min, even if slightly higher than in the absence of insulin, was not significantly different (1027 ± 219 vs 732 ± 109 for treated and untreated pancreata, respectively).

Discussion

Our experiments show an hypersensitivity to arginine of both B- and D-cells of pancreata from STZ-diabetic adult rats.
Arginine is well known to stimulate hormones from the normal pancreas (17, 18). However, in our experiments with normal rat pancreas, no stimulatory effect of arginine was observed on insulin secretion. This is probably due to the low glucose concentration used: 2.8 mmol/l. In fact, experiments previously performed in our laboratory on isolated perfused rat pancreas showed that arginine (5 mmol/l) increased insulin secretion in the presence of 8.3 mmol/l glucose (unpublished data). In contrast, in STZ-diabetic rat pancreas, arginine in the presence of 2.8 mmol/l glucose induced a paradoxical increase in insulin release. This finding can be compared with the results obtained by Giroix et al. (3) and Leahy et al. (4), although our experimental model is different, since in their experiments the rats were made diabetic as neonates.

Considering the effects of arginine on somatostatin secretion, our experiments show that in normal rat pancreas arginine did not stimulate somatostatin. In contrast, under the same conditions arginine stimulated somatostatin secretion from diabetic rat pancreas, thus indicating an hypersensitivity of the D-cells to this amino acid in STZ-diabetic rats. This observation is not in agreement with a previous report of Hermansen et al. (19); these authors found no difference in the pattern of somatostatin response to arginine in normal and STZ-diabetic dog pancreas. This discrepancy could be due to species difference or to different experimental conditions; indeed, an important difference, which must be underlined is that the glucose concentration is 2-fold higher in Hermansen’s experiments than in ours. Furthermore, it should be pointed out that in addition to D-cell hypersensitivity to arginine, the basal output of somatostatin from these STZ-diabetic rat pancreata was higher when compared with normal rats; this is in agreement with the results obtained in vitro by Hara et al. (8) and Gerich (20) who reported abnormalities in the number and function of the pancreatic D-cells during alloxan or STZ-diaetes. The high plasma somatostatin level observed in STZ-diabetic rats by Kazumi et al. (21) is also noted in our experiments. The results obtained with our experimental model can be put together with the observation by Skare et al. (22) that plasma SRIF is high in patients with insulin-dependent diabetes mellitus without residual B-cell function. However, in vivo experiments performed in normal (23) and alloxan-diabetic dogs (24) showed that circulating somatostatin levels do not reflect the secretory activity of the pancreatic D-cells. The experiments performed with insulin in diabetic rat pancreas show that the hypersensitivity of diabetic D-cells to arginine was not suppressed by the acute administration of insulin. So, even if the mechanism involved remains to be determined, a rapid direct action of insulin does not appear to be implicated.

In conclusion, our experiments show that not only the B-cells, but also the D-cells are hypersensitive to arginine in pancreata from STZ-diabetic adult rats.

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References

