Relative Hypersecretion of Amylin to Insulin From Rat Pancreas After Neonatal STZ Treatment

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With isolated perfused pancreases from normal and diabetic model rats, we studied alterations of the secretion of islet amyloid polypeptide, or amylin, which has been recently identified as a major component of amyloid deposits in the pancreatic islets of patients with non-insulin-dependent diabetes mellitus. Neonatal (n) Wistar-King albino rats given streptozocin (STZ) on the 2nd (n2STZ) or 5th (n5STZ) neonatal day exhibited moderate and marked elevations, respectively, of plasma glucose and HbA_1 as adults compared with control rats given the vehicle. The release of amylin from the perfused pancreases in response to glucose and arginine paralleled that of insulin in all three groups. However, the molar ratio of secreted amylin to insulin in response to 16.7 mM glucose by n5STZ pancreases (6.55 ± 0.71%) was significantly greater than that for either n2STZ (1.71 ± 0.24%, $P < 0.05$) or the control (0.60 ± 0.03%, $P < 0.05$) pancreases. The secreted amylin-insulin ratio of n2STZ pancreases also was significantly greater than that of the controls ($P < 0.05$). The increased amylin-insulin molar ratios of both n2STZ and n5STZ pancreases also occurred during infusions of 33.3 mM glucose and 10 mM arginine. These findings suggest that amylin secretion may be preserved in diabetic rats with reduced β-cell mass and that hyperglycemia may increase amylin production independently of that of insulin, which may be significant in the pathogenesis of non-insulin-dependent diabetes mellitus. *Diabetes* 41:723–27, 1992

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Interstitial deposits of amyloid within pancreatic islets have been considered a significant and specific pathological feature of non-insulin-dependent diabetes mellitus (NIDDM) (1,2). Several years ago, a major component of the islet amyloid deposits was characterized and named islet amyloid polypeptide (3), or amylin (4). This protein is a 37–amino acid peptide and has >40% amino acid sequence identity with calcitonin gene–related peptides (3,4). Genomic and cDNA studies have shown that human and rat amylin, like other neuroendocrine peptides, are generated by proteolytic processing, specifically from 89– and 93–amino acid prepro-amylin molecules (5,6). Ultrastructural studies with immunogold staining have shown that amylin colocalizes with insulin in the secretory granules of pancreatic β-cells (7,8). We and others have shown that amylin is cosecreted with insulin from isolated perfused rat pancreases in response to glucose and other insulin secretagogues (9,10). Amylin may be a new pancreatic hormone and a significant component of the islet amyloid deposits; however, its physiological action has not been fully characterized. A restraining action against insulin (11,12) and an inhibitory effect on insulin secretion (13) have been observed only at pharmacological doses. There also has been no report of amylin mutants causing islet amyloid deposition in NIDDM patients (14). Therefore, it seems likely that overproduction of amylin accounts for the process of amyloid deposition in pancreatic islets in NIDDM. In this study, with isolated perfused rat pancreases, we investigated alterations of amylin secretion in a NIDDM model produced by neonatal administration of streptozocin (STZ) (15,16).

RESEARCH DESIGN AND METHODS

Newborn Wistar-King albino rats (Institute of Experimental Animals, Kyushu University, Fukuoka, Japan) were

TABLE 1
Characteristics of control and diabetic rats given streptozocin (STZ) on the 2nd (n2STZ) and 5th (n5STZ) days after birth

	n	Body weight (g)	Fasting plasma insulin (pM)	Plasma glucose (mM)	HbA_1 (%)
Control	9	437 ± 16	448 ± 92	9.23 ± 0.42	4.9 ± 0.1
n2STZ	10	372 ± 7*	202 ± 40†	13.35 ± 1.09*	9.9 ± 1.2*
n5STZ	8	237 ± 8*‡	75 ± 8*§	25.49 ± 1.44*‡	15.4 ± 0.7*‡

Values are means ± SE.
Blood was taken by tail snipping in fed state at 16 wk of age for measurements of plasma glucose and HbA_1 levels.
*$P < 0.01$, †$P < 0.05$, vs. control.
‡$P < 0.01$, §$P < 0.05$, vs. n2STZ.

injected with 90 mg/kg STZ i.p. (Sigma, St. Louis, MO) dissolved in 0.05 M citrate buffer (pH 4.5) on the 2nd day of life (n2STZ) or with 80 mg/kg STZ i.p. on the 5th day of life (n5STZ) (15,16). Control newborn rats received only citrate buffer. The rats were weaned on 30 days of age and were then allowed to feed ad libitum on standard laboratory chow. At 16 wk of age, blood was collected from fed rats by tail snip for quantification of plasma glucose (PG) and glycosylated hemoglobin (GHb). Levels of PG were determined by a Beckman Glucose Analyzer II, and levels of GHb were measured by affinity chromatography (17). Both n2STZ and n5STZ rats with hyperglycemia >13.9 mM in the fed state at 16 wk of age were used for perfusion experiments. Just before isolation of the pancreas, 2 ml blood were taken from the aorta for measurements of insulin levels.

Pancreas perfusion. Male rats 16–20 wk of age were used for all experiments. Pancreases were isolated and perfused by the method of Grodsky and Fanska (18) with minor modifications (19). After an overnight fast, rats were anesthetized by injection of 50 mg/kg pentobarbital sodium i.p. The pancreas and adjacent proximal portion of the duodenum were isolated, and the celiac trunk and portal vein were cannulated. The organs were perfused at a constant flow of 3.6 ml/min by a peristaltic pump with a Krebs-Ringer bicarbonate buffer supplemented with 4.5% dextran T–70 (Pharmacia, Uppsala, Sweden), 1% bovine serum albumin (Sigma), and 5 mM each of pyruvate, fumarate, and glutamate. Glucose concentration of the basal perfusate was 5.6 mM. The perfusion medium was oxygenated with a mixture of 95% O_2 and 5% CO_2 and maintained at 37°C. Glucose and arginine were each dissolved in perfusion medium and added to the circulating perfusate through a sidearm infusion pump after an equilibration period of 20 min. One-minute aliquots of portal vein effluent were collected in chilled tubes containing 0.4 ml EDTA-benzamidine mixture (0.03 and 0.3 M, respectively) and stored until assay at −20°C.

Amylin and insulin radioimmunoassay (RIA). Effluent amylin concentration was determined by RIA (20). Specific antisera to rat amylin and ^{125}I-labeled rat amylin were purchased from Peninsula (Belmont, CA). RIA was performed by a double-antibody method with rat amylin standards diluted in perfusion medium containing 5.6 mM glucose. The minimum sensitivity of the assay was 2.6 pM. Within- and between-assay coefficients of variation (c.v.) were 8.9 and 9.1%, respectively. The curve of serial dilutions of a perfusate sample paralleled the standard curve and the c.v. of the parallelism was 5.1%.

Effluent insulin concentration was measured by RIA with a kit purchased from Dainabot (Tokyo) (10). Rat insulin standards (Novo, Copenhagen) diluted in perfusion medium were used. The curve of serial dilutions of a perfusate sample paralleled the standard curve.

Statistical analysis. Data are means ± SE. Total amylin and insulin secretion per pancreas was calculated from the area under the curve and converted to moles per minute. Statistical analysis was done by general analysis of variance to compare multiple groups, and significant statistical differences were determined by Scheffé's test with a probability level of <0.05.

RESULTS

The levels of PG and GHb of both n2STZ and n5STZ rats were significantly higher than those of controls (Table 1). In contrast, the body weight and fasting plasma insulin levels of both n2STZ and n5STZ rats were significantly lower than those of the controls. n5STZ rats showed the highest values of PG and GHb among the three groups and the lowest values of body weight and fasting plasma insulin.

The responses of amylin and insulin to glucose and arginine stimuli were compared among control, n2STZ, and n5STZ rat pancreases. In control rats, the infusion of either 16.7 mM glucose or 10 mM arginine elicited a biphasic release of amylin from the perfused pancreases in parallel with insulin release (Fig. 1). In n2STZ rat pancreases, 16.7 mM glucose and 10 mM arginine also caused a biphasic release of amylin and insulin. The secretion of amylin induced by 16.7 mM glucose was the same as that of controls, whereas the secretion of insulin was significantly less ($P < 0.01$) (Table 2). The secretion of amylin induced by 10 mM arginine was significantly greater than that of controls ($P < 0.01$), whereas the secretion of insulin did not differ significantly from that of controls (Table 2). Thus, the secreted amylin-insulin molar ratio by n2STZ rat pancreases was increased more than twofold as compared with controls (Table 2).

In pancreases from n5STZ rats, the infusion of 16.7 mM glucose caused minimal insulin secretion, whereas the infusion of 10 mM arginine elicited a significant insulin secretion (Fig. 1). The level of amylin secreted during perfusion with control buffer, which included 5.6 mM glucose, was significantly higher than that of controls (24.4 ± 0.9 vs. 1.1 ± 0.8 pM, $P < 0.01$), whereas the level of insulin during basal perfusion did not differ significantly from that of controls. As a result, the secre-

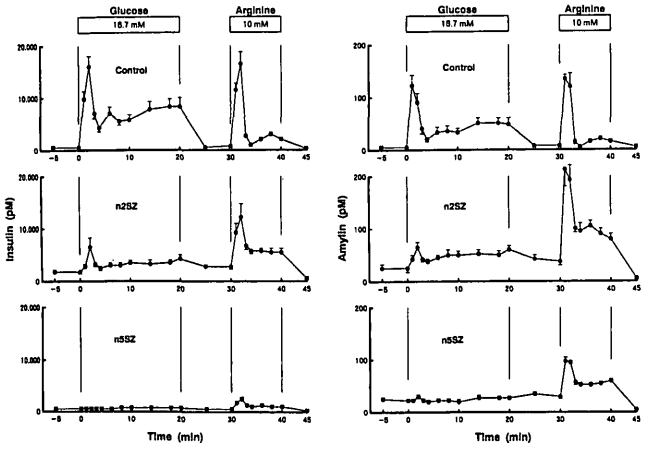

FIG. 1. Release of amylin and insulin from isolated perfused pancreas of control and diabetic model rats in response to 16.7 mM glucose and 10 mM arginine. n2STZ, rats given streptozocin on the 2nd day after birth; n5SZ, rats given streptozocin on the 5th day after birth. All perfused pancreases were from adult rats. The basal perfusates contained 5.6 mM glucose and 5 mM each of pyruvate, fumarate, and glutamate. Data are means ± SE of 4–6 experiments.

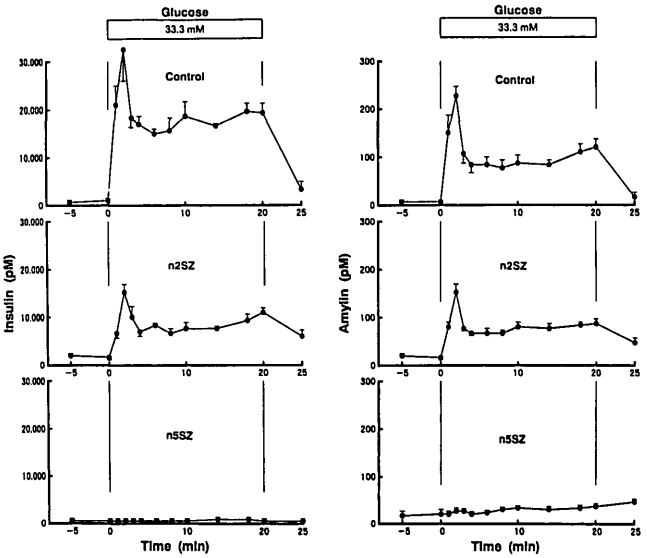

FIG. 2. Release of amylin and insulin from isolated perfused pancreas of control and diabetic model rats in response to 33.3 mM glucose. n2STZ, rats given streptozocin on the 2nd day after birth; n5STZ, rats given streptozocin on the 5th day after birth. All perfused pancreases were from adult rats. The basal perfusates contained 5.6 mM glucose and 5 mM each of pyruvate, fumarate, and glutamate. Data are means ± SE of 4 experiments.

tion of amylin induced by 16.7 mM glucose in n5STZ rat pancreases did not differ significantly from that of controls, whereas the secretion of insulin was markedly reduced ($P < 0.01$) (Table 2). Additionally, the secretion of amylin induced by 10 mM arginine in n5STZ rat pancreases was significantly greater than that of controls, whereas the secretion of insulin was significantly less ($P < 0.01$) (Table 2). Thus, the secreted amylin-insulin molar ratio in n5STZ rat pancreases was increased by 10-fold over controls (Table 2).

Similar alterations in the relationship between amylin and insulin secretion among control, n2STZ, and n5STZ rats occurred with the infusion of a higher concentration of glucose. Insulin release during the infusion of 33.3 mM glucose was significantly decreased in n2STZ rat pancreases and further decreased in n5STZ rat pancreases, as with 16.7 mM glucose infusion. However, amylin release during the infusion of 33.3 mM glucose in n2STZ rat pancreases did not differ significantly from that in controls (Fig. 2; Table 2). As a result, the secreted amylin-insulin molar ratio during the infusion of 33.3 mM glucose increased in order from control to n2STZ to n5STZ rat pancreas, as it did with infusions of 16.7 mM glucose and 10 mM arginine (Table 2).

DISCUSSION

We previously demonstrated secretion of both amylin and insulin from perfused normal rat pancreases in response to glucose and other insulin secretagogues (10,21). In this study, we investigated alterations of

TABLE 2
Secretion rates of amylin and insulin induced by 16.7 and 33.3 mM glucose, respectively, and 10 mM arginine from isolated perfused pancreas of normal and diabetic rats given streptozocin (STZ) on the 2nd (n2STZ) or 5th (n5STZ) day after birth

Secretagogue (mM)	*n*	Amylin (fmol/min)	Insulin (pmol/min)	Amylin-insulin molar ratio (%)
Glucose (16.7 mM)				
Control	5	186.2 ± 35.1	30.13 ± 4.28	0.60 ± 0.03
n2STZ	6	187.0 ± 25.0	12.38 ± 2.39‡	1.71 ± 0.24†
n5STZ	4	96.7 ± 9.9†	1.56 ± 0.29‡§	6.55 ± 0.71†‡
Arginine (10 mM)				
Control	5	145.5 ± 17.9	17.72 ± 1.39	0.82 ± 0.09
n2STZ	6	452.9 ± 48.4‡	26.07 ± 3.84	1.87 ± 0.21‡
n5STZ	4	252.8 ± 7.4‡†	3.99 ± 0.28‡§	6.41 ± 0.40*†‡
Glucose (33.3 mM)				
Control	4	408.7 ± 64.0	74.84 ± 8.83	0.54 ± 0.04
n2STZ	4	319.5 ± 30.3	35.41 ± 1.60‡	0.90 ± 0.08‡
n5STZ	4	120.2 ± 7.3†§	2.06 ± 0.34‡§	6.11 ± 0.62‡§

Values are means ± SE of several experiments. Total secretion of amylin and insulin during the infusion of each secretagogue was converted to secretion rate per minute and expressed as moles per minute.
*$P < 0.05$, ‡$P < 0.01$, vs. control.
†$P < 0.05$, §$P < 0.01$, vs. n2STZ.

amylin secretion in the diabetic state. For these experiments, rats were made diabetic by administering STZ during the neonatal period. Neonatal STZ (nSTZ) rats have been reported to have insulin secretion characteristics similar to human NIDDM patients with reduced insulin secretion, that is, secretion insensitive to glucose and hypersensitive to arginine (22,23). We used two varieties of this model by injecting STZ on two different neonatal days. Rats given STZ on the 5th day after birth (n5STZ) showed more severe hyperglycemia and hypoinsulinemia in vivo than rats given STZ on the 2nd day after birth (n2STZ), as previously reported (24). n2STZ rat pancreases showed a significant response of insulin to glucose that has not been found by others (22,23). The difference in the rat strain used or the 5 mM each of pyruvate, fumarate, and glutamate in the perfusion medium may account for this finding. Marked elevations of the basal secretion of both amylin and insulin by n2STZ pancreases and of amylin by n5STZ pancreases may also be because of the background of pyruvate, fumarate, and glutamate.

Linked secretion of amylin and insulin is also evident in both n2STZ and n5STZ rat pancreases as it is in controls, suggesting that amylin may be copackaged with insulin even in the secretory granules of nSTZ pancreatic β-cells. However, the relative amount of secreted amylin to insulin is dramatically increased in nSTZ diabetic rats. The secreted amylin-insulin molar ratio was increased by twofold in n2STZ rats and by tenfold in n5STZ rats compared with controls. Thus, as the degree of hyperglycemia and hypoinsulinemia became more severe, the amylin-insulin molar ratio increased. These results indicate that amylin secretion is preserved relative to insulin secretion in nSTZ rats, which have a reduced number of β-cells. This suggests that amylin may be hypersecreted relative to insulin in NIDDM, whereas insulin secretion is reduced. Ogawa et al. (9) reported that rats given STZ in adults exhibited a selective impairment of amylin secretion. Our results differ from this, probably because of the time of STZ injection. The sensitivity of the amylin RIA may also be a factor. We believe our results may reflect the effect of the diabetic state on amylin secretion in vivo rather than the toxic effect of STZ on pancreatic β-cells.

The mechanisms of the relative preservation of amylin secretion in nSTZ rat pancreases have not been clarified in this study, but hyperglycemia appears to increase amylin production out of proportion to that of insulin because the amylin-insulin molar ratio increased with increasing hyperglycemia. It has been reported that the ratio of amylin mRNA to insulin mRNA was increased in diabetic model rats produced by both dexamethasone and STZ treatment (25). Recently, Madsen et al. (26) reported that amylin and insulin expression were completely uncoupled in primary and transformed islet cells. These findings suggest that amylin expression may be regulated independently of insulin expression. Therefore, we speculate that hyperglycemia may increase amylin expression more than insulin expression and that this may be relevant to the pathophysiology of NIDDM. However, it may be necessary to take account of a lasting effect of STZ on the pancreatic β-cells especially of n5STZ rat, which may be more severely damaged than those of n2STZ rat by STZ treatment. Further studies are needed to clarify whether this relative hypersecretion of amylin in nSTZ rats may be corrected by lowering blood glucose levels.

Although islet amyloid has been thought to be a major pathological feature of NIDDM (2), the mechanism of amyloid deposition has not been clarified. Structurally abnormal or mutant proteins often cause amyloid deposition in various disease syndromes, accompanied by localized and systemic amyloidosis (27). However, neither abnormal amylin precursor sequences in NIDDM patients nor linkage of the amylin gene to NIDDM has been reported (14,28). Therefore, abnormal secretion of amylin and local conditions within the islets accelerating amyloid deposition may occur in NIDDM patients. Johnson et al. (29) reported increased islet amyloid polypeptide immunoreactivity in pancreatic β-cells of cats with impaired glucose tolerance. Our study suggests that relative hypersecretion of amylin may occur in NIDDM patients and that this alteration of amylin secretion may contribute to islet amyloid deposition.

The finding that amylin release parallels insulin release from both normal and diabetic perfused rat pancreases, despite the relative hypersecretion of amylin in nSTZ rats, indicates that the ratio of amylin to insulin in the β-cell secretory granules of nSTZ rats is increased. A higher proportion of amylin in secretory granules might result in a disturbance of the secretory process and an acceleration of β-cell dysfunction (30). In some NIDDM patients, fibrillar immunoreactive amyloid deposits have been found within the cytoplasm of β-cells (31). The relatively high content of amylin in secretory granules might contribute to this intracellular amyloid formation during the degradation of secretory granules (32).

In conclusion, relative hypersecretion of amylin to insulin occurs in isolated perfused pancreases of diabetic rats produced by neonatal treatment with STZ. These results suggest that amylin secretion in NIDDM patients may be preserved while their insulin secretion is reduced and that this relative hypersecretion of amylin may be linked to the pathophysiology of NIDDM.

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