Experimental NIDDM

Development of a New Model in Adult Rats Administered Streptozotocin and Nicotinamide

Pellegrino Masiello, Christophe Broca, René Gross, Michèle Roye, Michèle Manteghetti, Dominique Hillaire-Buys, Michela Novelli, and Gérard Ribes

We took advantage of the partial protection exerted by suitable dosages of nicotinamide against the β-cytotoxic effect of streptozotocin (STZ) to create a new experimental diabetic syndrome in adult rats that appears closer to NIDDM than other available animal models with regard to insulin responsiveness to glucose and sulfonylureas. Among the various dosages of nicotinamide tested in 3-month-old Wistar rats (100–350 mg/kg body wt), the dosage of 230 mg/kg, given intraperitoneally 15 min before STZ administration (65 mg/kg i.v.) yielded a maximum of animals with moderate and stable nonfasting hyperglycemia (155 ± 3 vs. 121 ± 3 mg/dl in controls; $P < 0.05$) and 40% preservation of pancreatic insulin stores. We also evaluated β-cell function both in vitro and in vivo 4–9 weeks after inducing diabetes. In the isolated perfused pancreas, insulin response to glucose elevation (5–11 mmol/l) was clearly present, although significantly reduced with respect to controls ($P < 0.01$). Moreover, the insulin response to tolbutamide (0.19 mmol/l) was similar to that observed in normal pancreases. Perfused pancreases from diabetic animals also exhibited a striking hypersensitivity to arginine infusion (7 mmol/l). In rats administered STZ plus nicotinamide, intravenous glucose tolerance tests revealed clear abnormalities in glucose tolerance and insulin responsiveness, which were interestingly reversed by tolbutamide administration (40 mg/kg i.v.). In conclusion, this novel NIDDM syndrome with reduced pancreatic insulin stores, which is similar to human NIDDM in that it has a significant response to glucose (although abnormal in kinetics) and preserved sensitivity to tolbutamide, may provide a particularly advantageous tool for pharmacological investigations of new insulinotropic agents. *Diabetes* 47:224–229, 1998

From the Istituto di Patologia Generale (P.M., M.N.), University of Pisa, Italy; the Laboratoire de Pharmacologie (UPRES EA 1677) (C.B., D.H.-B.), Faculté de Médecine; and UMR 9921 du Centre National de la Recherche Scientifique (C.B., R.G., M.R., M.M., G.R.), Montpellier, France.

Address correspondence and reprint requests to Dr. Pellegrino Masiello, Istituto di Patologia Generale, Via Roma, 55, Scuola Medica, I-56127, Pisa, Italy.

Received for publication 9 July 1997 and accepted in revised form 16 October 1997.

AUC, areas under the curve; GK rat, Goto-Kakizaki rat; IVGTT, intravenous glucose tolerance test; STZ, streptozotocin.

In various animal species, several syndromes resembling NIDDM either occur spontaneously or can be induced experimentally by different procedures (1). None of these syndromes is able to reproduce the complexity of human diabetes; nevertheless, each one can be very helpful for understanding at least some aspects of the pathogenesis and evolution of the disease and possibly testing new antidiabetic drugs.

It should be noticed that two well-recognized experimental models of NIDDM without associated obesity (i.e., partially pancreatectomized rats and rats subjected to a neonatal administration of streptozotocin [STZ]) are characterized by a substantial reduction in β-cell mass (2–4) that is considered to occur also in NIDDM patients (5,6). It has recently been reported that in a commonly used genetic model of NIDDM, the Goto-Kakizaki (GK) rat, β-cell mass depletion precedes the onset of metabolic abnormalities (7).

A common feature of the three above-mentioned animal diabetic syndromes is glucose insensitivity (8–11), which actually does not occur in the human disease; indeed, insulin responsiveness to glucose, although altered both qualitatively and quantitatively, is present in NIDDM (12,13). In rats neonatally administered STZ (n-STZ rats), another remarkable difference from NIDDM is the lack of insulin response to tolbutamide in the presence of glucose (14). In GK rats, a stimulatory effect on insulin release from isolated islets has been shown after incubation with glucose and gliclazide (15).

Because the known experimental models of NIDDM appear unsatisfactory with regard to the insulin response to glucose and sulfonylureas, we considered it worthwhile to try to establish a new alternative experimental diabetic syndrome with reduced pancreatic insulin stores that could maintain, to some extent, responsiveness to both glucose and sulfonylureas and therefore be more suitable for pharmacological research. The idea arose from our previous experience on the protective effects of nicotinamide and 3-aminobenzamide against STZ-induced diabetes in the adult rat (16,17) and from the knowledge that the degree of protection was correlated to the residual pancreatic insulin content and could be modulated by administration of different dosages of the protective agent (16).

The present study dealt with the establishment of such a diabetic syndrome in adult rats administered STZ and partially protected with suitable dosages of nicotinamide. This syndrome shares a number of features with NIDDM, and is char-

acterized by stable moderate hyperglycemia, glucose intolerance, altered but significant glucose-stimulated insulin secretion, and in vivo and in vitro responsiveness to tolbutamide.

RESEARCH DESIGN AND METHODS

Animals and treatment. Groups of 10-week-old male Wistar rats (IFFA-Credo, L'Arbresle, France), weighing 220–230 g received various intraperitoneally administered dosages (range 100–350 mg/kg body wt) of nicotinamide (Sigma, St. Louis, MO) dissolved in saline 15 min before an intravenous administration of 65 mg/kg STZ (Zanosar; a gift from Upjohn, Paris, France) dissolved in saline immediately before use. Each set of animals administered STZ plus nicotinamide was paralleled by two other groups, one receiving STZ and the vehicle of nicotinamide (unprotected diabetic rats) and the other, the vehicles of both substances (controls). Blood was sampled from the tail vessels of conscious nonfasting animals in EDTA-containing chilled tubes. After centrifugation at 4°C, plasma was stored at –20°C until assayed.

Intravenous glucose tolerance test. Glucose (0.5 g/kg) was given as a 30% solution through the tail vein of conscious nonfasting animals. Blood samples were collected sequentially from the tail vein before and 5, 15, 30, and 60 min after the sugar injection. Tolbutamide (40 mg/kg) was administered intravenously together with glucose; samples were collected before and 2, 6, 15, 30, and 60 min after the injection. After centrifugation at 4°C, 10 µl of plasma were immediately used for glucose determination and the rest was kept at –20°C until insulin radioimmunoassay.

Isolated perfused pancreas technique. Pancreases were isolated from rats anesthetized with sodium pentobarbitone (60 mg/kg i.p.) 4–8 weeks after diabetes was induced. The technique of Loubatières et al. (18) was used to isolate the pancreas from neighboring tissues. The organ was then transferred into a plastic chamber maintained at 37°C. The perfusion medium (Krebs-Ringer bicarbonate buffer containing 2 g/l bovine serum albumin and 5 mmol/l glucose) was continuously bubbled with a mixture of 95% O_2/5% CO_2. Infusion pressure was adjusted to provide a pancreatic outflow of 2.5 ml/min. A 30-min equilibration period was allowed before taking the first sample. Two more samples were collected 10 and 15 min later. To test β-cell function, glucose concentration was increased from 5 to 11 mmol/l, and 0.19 mmol/l tolbutamide, a reference sulfonylurea, was subsequently infused in the presence of 5 mmol/l glucose. Samples were taken every minute for 5 min after each stimulation, then every 5 min for 15 min. In other perfusion experiments, L-arginine hydrochloride (Sigma, St. Louis, MO) was infused for 20 min in the presence of 2.8 mmol/l glucose. All samples were collected for 1 min, allowing pancreatic effluent output to be determined, and were immediately frozen for insulin assay. The insulin output rate was calculated as the hormone concentration (ng/ml) in the effluent × the corresponding flow rate (ml/min).

Extraction of pancreatic insulin. At the end of the 95-min perfusion period, the pancreas was carefully wiped on filter paper, weighed, and homogenized in a cold mixture of 0.7 mol/l HCl:ethanol (1:3, vol/vol), which was kept for 48 h at 4°C. After centrifugation and separation of the supernatant, the pellet was extracted again with acidified ethanol for 24 h at 4°C. The supernatant obtained after centrifugation was pooled with the previous one and kept at –20°C until assayed.

Assays. Insulin concentrations in plasma and pancreatic extracts were measured by the radioimmunological method of Herbert et al. (19) using an antibody supplied by ICN Biochemicals (Costa Mesa, CA) and rat insulin (Novo, Copenhagen, Denmark) as standard. The intra- and interassay coefficients of variation were 9 and 13.5%, respectively. The analytical sensitivity was 0.10 ng/ml. Plasma glucose levels were determined by the glucose oxidase method using commercial kits (Boehringer, Mannheim, Germany).

Expression of data and statistical methods. Insulin output from the isolated perfused pancreas is expressed as ng/min and as areas under the curve (AUCs) during infusion of the tested substances. After the intravenous glucose tolerance test (IVGTT), glucose disappearance rates were calculated from the slope of the logarithm of the postload plasma glucose concentrations between the peak value (at 2 or 5 min) and the value at 30 min (coefficient K) and were expressed as percentage per minute. Data are given as means ± SE and have been statistically evaluated by analysis of variance with the multiple comparison test of Newman-Keuls.

RESULTS

Characteristics of animals. Table 1 shows the main metabolic features of the rats used in this study 4 weeks after the administration of STZ and the dosages of nicotinamide used. At the lower dosage (100 mg/kg), nicotinamide exerted only minor protection against the dramatic effect of STZ on body weight, glycemia, and insulinemia. At 180 mg/kg, nicotinamide largely prevented STZ-induced body weight loss, hyperglycemia, and hypoinsulinemia. With nicotinamide at 230 mg/kg, only blood glucose concentrations were significantly different ($P < 0.05$) from control values. Finally, the highest nicotinamide dosage (350 mg/kg) fully prevented STZ-induced alterations.

Insulin release from isolated perfused pancreas

Insulin response to glucose or tolbutamide. Insulin output in the isolated perfused pancreas stimulated by a rise in glucose concentration from 5 to 11 mmol/l glucose and subsequently by 0.19 mmol/l tolbutamide is shown in Figs. 1 and 2. In Fig. 1 it can be observed that perfused pancreases of STZ-induced diabetic rats were unresponsive to both stimuli, as were the pancreases of rats given STZ plus 100 mg/kg nicotinamide. As for the rats administered STZ plus 180 mg/kg nicotinamide, the glucose-stimulated first phase of insulin release peaked after 2 min at 40% of control values, whereas the second phase was more clearly blunted. During overall glucose stimulation (20 min), the AUC was 78 ± 11 vs. 285 ± 35 ng for control pancreases ($P < 0.01$). In the presence of 5 mmol/l glucose, tolbutamide elicited an insulin response that attained 54% of control values. Indeed, after stimulation with this sulfonylurea (10 min), AUC was 61 ± 17 ng vs. 112 ± 17 ng for control pancreases (NS).

Figure 2 shows the results obtained in a large series of rats administered STZ plus nicotinamide 230 mg/kg and appropriate controls. Basal insulin release at 5 mmol/l glucose was higher in treated animals than in controls. Thus at 45 min, insulin output was 3.7 ± 0.5 vs. 0.8 ± 0.2 ng/min of normal pancreases ($P < 0.01$). The first phase of glucose-stimulated insulin release peaked at 74% of control values, whereas the

TABLE 1
Body weight and basal plasma glucose and insulin levels in nonfasting Wistar rats 4 weeks after administration of STZ and various dosages of nicotinamide

	Controls	STZ	STZ + 100 mg/kg nicotinamide	STZ + 180 mg/kg nicotinamide	STZ + 230 mg/kg nicotinamide	STZ + 350 mg/kg nicotinamide
Body weight (g)	339 ± 6	245 ± 9†	293 ± 4†	313 ± 6†	333 ± 5	337 ± 3
Plasma glucose (mg/dl)	121 ± 3	639 ± 19†	568 ± 21†	189 ± 19†	155 ± 3*	123 ± 2
Plasma insulin (ng/ml)	2.20 ± 0.20	0.12 ± 0.03†	0.30 ± 0.03†	1.56 ± 0.17†	1.94 ± 0.14	2.56 ± 0.21

Data are means ± SE of 6–16 observations. For some groups of rats, results from different experimental series were pooled. *$P <$ 0.05 vs. control values; †$P < 0.01$ vs. control values.

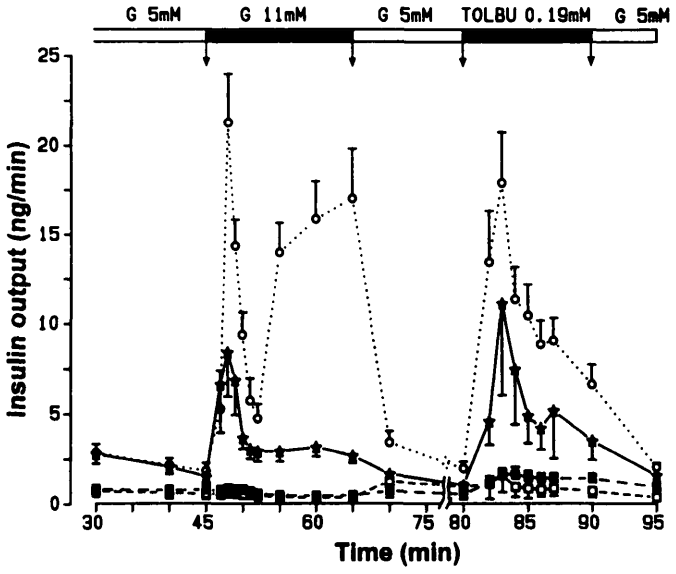

FIG. 1. Glucose (G)- and tolbutamide (TOLBU)-stimulated insulin output from the isolated perfused pancreases of rats administered STZ and various dosages of nicotinamide. Pancreas perfusions were performed 3–5 weeks after diabetes induction. □, STZ alone; ■, STZ plus 100 mg/kg nicotinamide; ★, STZ plus 180 mg/kg nicotinamide; ○, controls. Data are means ± SE of five to seven observations in each group.

second phase was evidently defective, resulting in a 20-min AUC of 134 ± 11 vs. 210 ± 26 ng for control pancreases ($P < 0.01$). Interestingly, tolbutamide-induced secretion was close to that of controls, with 10-min AUCs being 89 ± 8 and 71 ± 16 ng, respectively (NS). The in vitro insulin response to glucose and tolbutamide of rats given 350 mg/kg nicotinamide was superimposable on that of controls (data not shown).

Pancreatic insulin stores. Unlike the 100 mg/kg dosage, the nicotinamide dosages of 180 and 230 mg/kg were able to limit substantially the dramatic fall in pancreatic insulin content caused by STZ alone (Fig. 3). In particular, the protective treatment with 230 mg/kg nicotinamide resulted in the preservation of 40% of normal pancreatic insulin stores. Finally, the

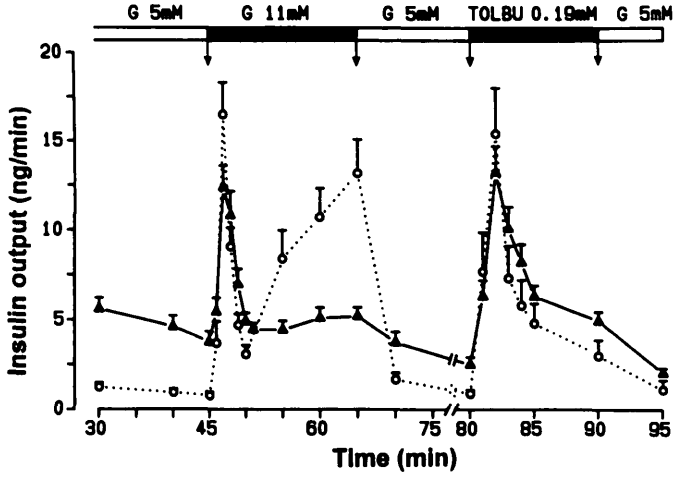

FIG. 2. Glucose (G)- and tolbutamide (TOLBU)-stimulated insulin output from the isolated perfused pancreases of rats administered STZ plus 230 mg/kg nicotinamide (▲) and controls (○). Pancreas perfusions were performed 5–8 weeks after diabetes induction. Data are means ± SE of five to eight observations.

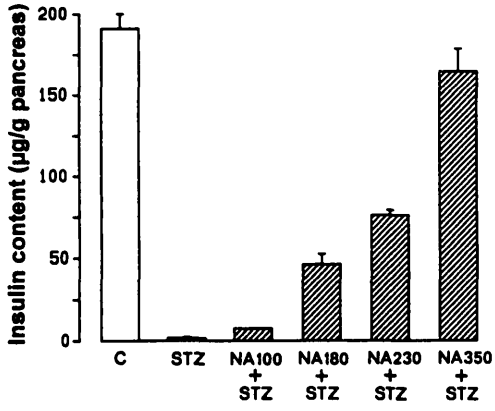

FIG. 3. Effects of various dosages of nicotinamide (NA) on the STZ-induced reduction of pancreatic insulin content in Wistar rats. ■, rats administered STZ alone; ▨, rats administered STZ plus the indicated dosages (mg/kg) of NA; □, control (C) rats. Data are means ± SE of five to seven observations.

insulin content of rats protected with 350 mg/kg nicotinamide was not significantly different from that of controls.

Insulin response to arginine. In the presence of 2.8 mmol/l glucose, arginine (7 mmol/l) did not significantly affect insulin output from normal pancreases, whereas it induced a remarkable biphasic response from pancreases of rats administered STZ plus 230 mg/kg nicotinamide (Fig. 4). This abnormally high response was characterized by a striking first phase, peaking after 2 min at 26.2 ± 2.0 vs. 0.5 ± 0.2 ng/min in controls ($P < 0.01$), and a much lower second phase.

In vivo experiments

Basal plasma glucose and insulin levels. The less severe reduction in insulin stores obtained with 230 mg/kg nicotinamide prompted us to use this dosage in our in vivo experiment. Figure 5 shows the time course of basal plasma glucose concentrations of nonfasting rats administered STZ

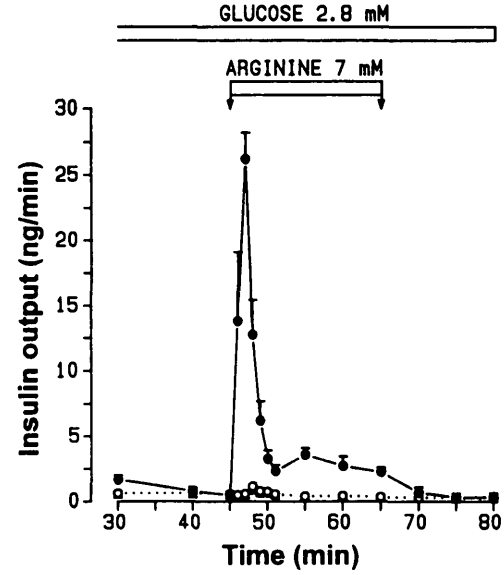

FIG. 4. Effect of 7 mmol/l arginine in the presence of 2.8 mmol/l glucose on insulin secretion from the isolated perfused pancreas of rats administered STZ plus 230 mg/kg nicotinamide (●) and controls (○). Data are means ± SE of four observations.

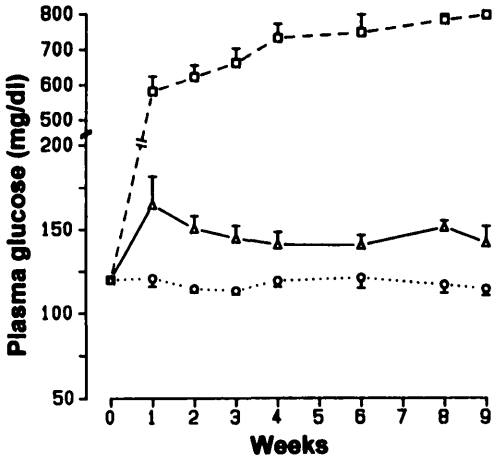

FIG. 5. Time course of plasma glucose levels of rats administered STZ plus 230 mg/kg nicotinamide (△) versus control (○) and STZ-administered (□) animals. Data are means ± SE of five to six observations.

plus 230 mg/kg nicotinamide compared with control and unprotected animals administered STZ. Interestingly, the moderate hyperglycemia induced by administration of STZ plus nicotinamide remained fairly stable at 2–9 weeks after the treatment. During this period, basal plasma insulin levels in these rats (1.9 ± 0.3, 2.1 ± 0.4, and 2.2 ± 0.6 ng/ml at 2, 4, and 9 weeks, respectively) were not significantly different from those of control animals (2.2 ± 0.2, 1.8 ± 0.3, and 1.9 ± 0.4 ng/ml at 2, 4, and 9 weeks, respectively).

IVGTTs. As shown in Fig. 6, after an intravenous glucose injection (0.5 g/kg) in normal rats, postload plasma glucose and insulin levels progressively returned to basal values within approximately 30 min. In rats administered STZ plus nicotinamide, IVGTTs performed 4 weeks after induction of diabetes revealed a marked glucose intolerance. Indeed, glycemic levels (Fig. 6*A*) attained 320 ± 10 mg/dl at 5 min after glucose injection, decreasing slowly thereafter (at 60 min, glycemia was 206 ± 7 mg/dl). As expected, glucose administration in these animals caused a limited increase of circulating insulin levels that, however, remained slightly higher than basal values for 60 min (Fig. 6*B*). Interestingly, IVGTTs repeated in the same animals 9 weeks after diabetes induction showed the same abnormalities in insulin responsiveness and the same degree of glucose intolerance as earlier (Fig. 6). Indeed, the postload glucose disappearance rates between 5 and 30 min in rats administered STZ plus nicotinamide were similar at 4 and 9 weeks after treatment (K = 1.50 ± 0.17 and 1.18 ± 0.10%, respectively) and both were markedly lower ($P < 0.01$) than that observed in untreated controls (K = 2.76 ± 0.40%).

To evaluate the effectiveness of sulfonylurea compounds to correct glucose intolerance in this experimental diabetic syndrome, glucose (0.5 g/kg i.v.) and tolbutamide (40 mg/kg i.v.) were simultaneously administered in rats administered STZ plus 230 mg/kg nicotinamide and appropriate controls (Fig. 7). As is seen in Fig. 7*B*, tolbutamide induced a remarkable and sustained increase in circulating insulin levels in diabetic animals compared with that obtained in response to glucose alone. As a consequence, a substantial improvement of glucose tolerance occurred in this group of rats (Fig. 7*A*), with plasma glucose returning to preload levels within 30 min and continuing to go down at 60 min. The postload glucose disappearance

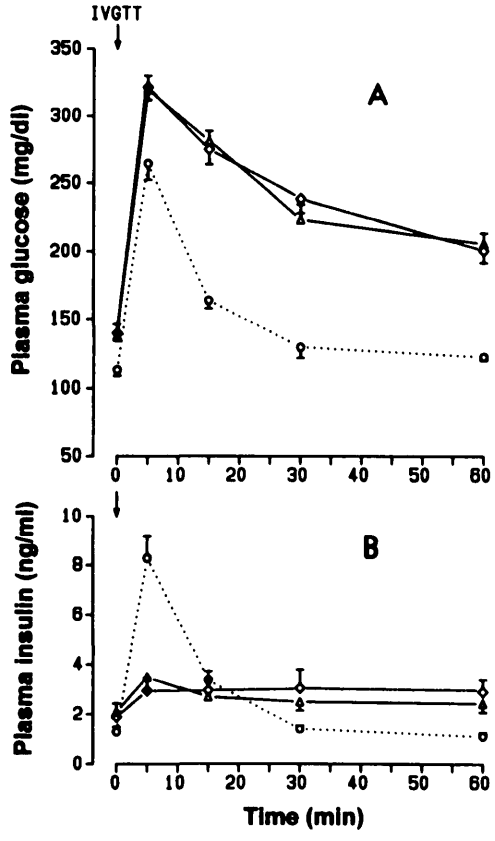

FIG. 6. Plasma glucose (*A*) and insulin (*B*) levels during IVGTT performed in the same individual rats at 4 (△) and 9 (◇) weeks after being administered STZ plus 230 mg/kg nicotinamide. Glucose was administered at a dosage of 0.5 g/kg. ○·····○, pooled data of corresponding control rats. Data are means ± SE of four observations in treated rats and eight observations in controls.

rate between 2 and 30 min in these diabetic rats was significantly higher in the presence than in the absence of tolbutamide (K = 2.67 ± 0.44 vs. 1.03 ± 0.27%; $P < 0.05$), and was not significantly different from that observed in tolbutamide-treated normal rats (K = 3.77 ± 0.36%). Nevertheless, it should be noticed that the tolbutamide-induced elevation in plasma insulin concentrations was higher in control than in diabetic animals and led to hypoglycemia at 30 and 60 min (Fig. 7).

DISCUSSION

Our results clearly showed that the combined administration of STZ with suitable dosages of nicotinamide to adult rats leads to the development of an interesting novel diabetic syndrome, characterized by moderate and stable hyperglycemia and reduced pancreatic insulin stores (approximately 40% of normal). This experimental syndrome appears closer to human NIDDM than other commonly used animal models, at least with regard to insulin responsiveness to glucose and sulfonylureas. Therefore these diabetic animals could be useful in testing new pharmacological agents with potential insulinotropic action.

It is worthwhile to note that the protective effect of nicotinamide against STZ β-cytotoxicity is thought to be dependent on the preservation of the intracellular NAD pool accomplished by this compound (20,21). Indeed, on one side nicotinamide is a direct precursor of NAD, and on the other side is an inhibitor of poly(ADP-ribose) synthetase, an NAD-

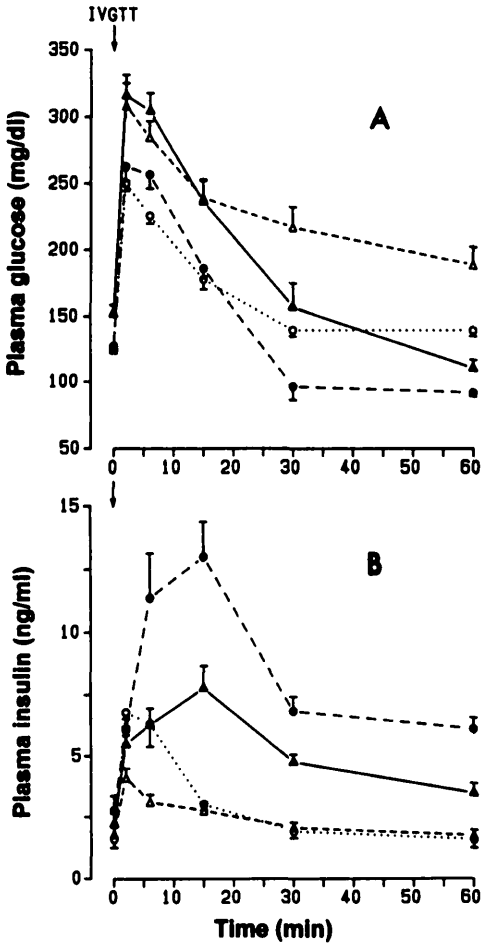

FIG. 7. Effects of tolbutamide on plasma glucose (*A*) and insulin (*B*) levels during IVGTT performed in rats administered STZ plus 230 mg/kg nicotinamide. Tolbutamide (40 mg/kg) was injected simultaneously with glucose (0.5 g/kg) by intravenous route 7–8 weeks after diabetes was induced. ○, controls given glucose alone; ●, controls given glucose plus tolbutamide; △, rats administered STZ plus nicotinamide and then given glucose alone; ▲, rats administered STZ plus nicotinamide and then given glucose plus tolbutamide. Data are means ± SE of four to six observations in each group.

consuming enzyme activated by STZ-induced DNA injury (21,22). The effectiveness of the protection depends on the relative dosages of STZ and nicotinamide (23). Thus, if the dosage of nicotinamide is high enough, STZ-administered rats can survive for a long time without any evidence of deficiency in insulin-secreting function; conversely, they develop functioning islet cell tumors after a long latency (24).

In the attempt to achieve a slight or moderate hyperglycemia in nonfasting animals without a profound depletion in pancreatic insulin stores, various protective dosages of nicotinamide (100–350 mg/kg) were tested in STZ-administered rats. Because of the higher residual content of pancreatic insulin (40% of control values, similar to that of n-STZ [4,25] and GK [7] rats) and the higher yield (~75%) of animals with glycemic levels above those of controls but <200 mg/dl, the nicotinamide dosage of 230 mg/kg appears to be the most appropriate one in our experimental conditions for future investigations; this observation is also based on in vitro functional studies. Indeed, the isolated perfused pancreases of animals administered STZ plus 230 mg/kg nicotinamide responded to an elevation of glucose concentration from 5 to 11 mmol/l, with a biphasic pattern of insulin secretion characterized by a prompt but reduced first phase (slightly smaller with nicotinamide at 180 mg/kg) and a markedly defective second phase. It should be noted that if the relative magnitude of the two phases is at variance with what is considered to occur in NIDDM, it nevertheless contrasts with the absence of in vitro glucose responsiveness reported for other animal models of NIDDM, such as n-STZ or GK rats (9,10), unless a prolonged period of glucose deprivation was imposed before high glucose (16–27 mmol/l) stimulation (10,25).

Most interestingly, rats administered STZ plus nicotinamide showed a well-preserved in vitro sensitivity to sulfonylureas, particularly those treated with 230 mg/kg nicotinamide, whose insulin response to 0.19 mmol/l tolbutamide in the presence of 5 mmol/l glucose was superimposable on that of controls. Such sensitivity to sulfonylureas, which is typical of NIDDM, was previously observed only for GK rats after static incubation of isolated islets with gliclazide (15), whereas in n-STZ rats it was reported in the absence of glucose only (9).

It must also be noted that in several (but not all) perfused pancreases of rats administered STZ plus nicotinamide (e.g., the large series given 230 mg/kg nicotinamide, which included several animals used 6–8 weeks after treatment), prestimulatory basal insulin output (at 5 mmol/l glucose) was unusually high compared with that of controls. This high basal release showed a clear tendency to decrease with perfusion time, as is also indicated by the values measured after the withdrawal of high glucose. A similarly high basal insulin release in perfused pancreas preparations also has been observed in GK (10) and n-STZ (8,26) rats. It is reported more often in animals with increased peripheral insulin resistance and is considered secondary to enhanced demand on β-cell secretion (26) and glycogen-derived endogenous glucose production by β-cells (27).

A striking feature in rats administered STZ plus nicotinamide is the marked hypersensitivity of β-cells to arginine; indeed, at glucose concentrations as low as 2.8 mmol/l, insulin release in response to 7 mmol/l arginine, a small amount in normal rats, was drastically increased in diabetic animals, as has also been reported for other animal models of NIDDM (8–10). The reason for such an exaggerated arginine effect remains unclear.

The present experimental model of diabetes in adult rats is of practical interest, given the facility with which diabetes can be induced in these animals and the short time it takes these animals to present with a great stability in their metabolic alterations, as indicated by the unchanged glycemia and responsiveness to IVGTT during the first 2 months after the induction of diabetes. Incidentally, it is noteworthy that in the adult rats receiving different low dosages of STZ to obtain various degrees of glucose intolerance or hyperglycemia, most displayed progressive increments in plasma glucose levels and deterioration of glucose tolerance in a few weeks (28).

IVGTT performed in animals administered STZ plus nicotinamide revealed a marked glucose intolerance, as expected. With regard to postload plasma insulin concentrations, an early small peak (higher at 2 than at 5 min) occurred, with a tendency to return to basal values more slowly than control rats did from their sharp insulin peak. This behavior is most likely indicative of an altered but still oper-

ative ability of residual β-cells to respond to glucose. These cells, however, maintain a much greater sensitivity to sulfonylurea stimulation, as can be argued from the effects of glucose plus tolbutamide intravenous loading, which strongly supports our in vitro data and implies that the defective insulin-secreting capabilities in these animals can be efficiently corrected by an appropriate stimulus. From this point of view, the analogy with human NIDDM is straightforward.

It is also noteworthy that after tolbutamide stimulation, the rate of glucose disappearance from the blood in the diabetic rats paralleled that of controls and resulted in normalization of glycemia. Nevertheless, the magnitude of tolbutamide-stimulated insulin secretion was much larger in control than in diabetic animals and led to hypoglycemia. By comparison, in both n-STZ (29) and GK (10) rats, plasma insulin release in response to intravenous glucose was lacking, and no comparable data about acute sulfonylurea administration in these two established NIDDM models are available in the literature.

In an attempt to explain the better capability of our diabetic animals administered STZ plus nicotinamide to maintain β-cell sensitivity to glucose and tolbutamide than n-STZ rats, we speculate that β-cells of adult rats protected by nicotinamide remain well differentiated and at least partially responsive to physiological and/or pharmacological stimuli. In n-STZ rats, most of the residual β-cell mass derives from a regeneration process (3,4), which could give rise to cell populations with reduced glucose sensitivity.

Finally, no clear-cut evidence of alteration of insulin action was observed in rats administered STZ plus nicotinamide in the present study. However, the possibility that insulin resistance develops in these animals is currently under investigation in our laboratory.

In summary, we have characterized in adult rats given STZ and appropriate protective dosages of nicotinamide a new diabetic syndrome with reduced pancreatic insulin stores that mimics some features of NIDDM not shared by other established animal models of the disease, namely, partial responsiveness to glucose (even though with different in vitro kinetics) and well-preserved sensitivity to sulfonylureas. Other advantages of this syndrome include *1*) the facility of the induction of diabetes, *2*) the high yield of mildly diabetic animals, and *3*) the stability of metabolic alterations.

Thus all these characteristics make rats administered STZ plus nicotinamide an attractive alternative to other genetic or acquired experimental models of NIDDM and suitable in particular for both acute and chronic pharmacological investigations of insulinotropic agents potentially useful for the treatment of NIDDM.

ACKNOWLEDGMENTS

The excellent technical assistance of Michel Tournier and Valérie Montesinos is gratefully acknowledged.

REFERENCES

1. Shafrir E: Diabetes in animals. In *Diabetes Mellitus: Theory and Practice.* Rifkin H, Porte D Jr, Eds. New York, NY, Elsevier, 1990, p. 299–340
2. Bonner-Weir S, Trent DF, Weir GC: Partial pancreatectomy in the rat and subsequent defect in glucose-induced insulin release. *J Clin Invest* 71:1544–1553, 1983
3. Weir GC, Leahy JL, Bonner-Weir S: Experimental reduction of beta-cell mass: implications for the pathogenesis of diabetes. *Diabetes Metab Rev* 2:125–161, 1986
4. Portha B, Blondel O, Serradas P, McEvoy R, Giroix M-H, Kergoat M, Bailbe D: The rat models of non-insulin dependent diabetes induced by neonatal streptozotocin. *Diabete Metab* 15:61–75, 1989
5. Stefan I, Orci L, Malaisse-Lagae F, Perrelet A, Patel Y, Unger RH: Quantitation of endocrine cell content in the pancreas of nondiabetic and diabetic humans. *Diabetes* 31:694–700, 1982
6. Klöppel G, Löhr M, Hablich K, Oberholzer M, Heitz PU: Islet pathology and pathogenesis of type I and type II diabetes revisited. *Surv Synth Pathol Res* 4:110–125, 1985
7. Movassat J, Saulner C, Portha B: Beta-cell mass depletion precedes the onset of hyperglycaemia in the GK rat, a genetic model of non-insulin-dependent diabetes mellitus. *Diabete Metab* 21:365–370, 1995
8. Leahy JL, Bonner-Weir S, Weir GC: Abnormal glucose regulation of insulin secretion in models of reduced B-cell mass. *Diabetes* 33:667–673, 1984
9. Giroix M-H, Portha B, Kergoat M, Bailbe D, Picon L: Glucose insensitivity and amino-acid hypersensitivity of insulin release in rats with non-insulin-dependent diabetes: a study with the perfused pancreas. *Diabetes* 32:445–451, 1983
10. Portha B, Serradas P, Bailbe D, Suzuki K, Goto Y, Giroix M-H: Beta-cell insensitivity to glucose in the GK rat, a spontaneous nonobese model for type 2 diabetes. *Diabetes* 40:486–491, 1991
11. Östenson CG, Khan A, Abdel-Halim SM, Guenifi A, Suzuki K, Goto Y, Efendic S: Abnormal insulin secretion and glucose metabolism in pancreatic islets from the spontaneously diabetic GK rat. *Diabetologia* 36:3–8, 1993
12. Cerasi E, Luft R: The plasma insulin response to glucose infusion in healthy subjects and in diabetes mellitus. *Acta Endocrinol* 55:278–304, 1967
13. Ward WK, Beard JC, Halter JB, Pfeiffer MA, Porte D Jr: Pathophysiology of insulin secretion in non-insulin-dependent diabetes mellitus. *Diabetes Care* 7:492–501, 1984
14. Weir GC, Clore ET, Zmachinski CJ, Bonner-Weir S: Islet secretion in a new experimental model for non-insulin-dependent diabetes. *Diabetes* 30:590–595, 1981
15. Giroix M-H, Vesco L, Portha B: Functional and metabolic perturbations in isolated pancreatic islets from the GK rat, a genetic model of noninsulin-dependent diabetes. *Endocrinology* 132:815–822, 1993
16. Masiello P, Locci-Cubeddu T, Frosina G, Bergamini E: Protective effect of 3-aminobenzamide, an inhibitor of poly (ADP-ribose) synthetase, against streptozotocin-induced diabetes. *Diabetologia* 28:683–686, 1985
17. Masiello P, Novelli M, Fierabracci V, Bergamini E: Protection by 3-aminobenzamide and nicotinamide against streptozotocin-induced beta-cell toxicity in vivo and in vitro. *Res Commun Chem Pathol Pharmacol* 69:17–32, 1990
18. Loubatières AL, Mariani MM, De Malbosc H, Ribes G, Chapal J: Etude expérimentale d'un nouveau sulfamide hypoglycémiant pariculièrement actif, le HB 419 ou glibenclamide. I. Action betacytotrope et insulino-sécrétrice. *Diabetologia* 5:1–10, 1969
19. Herbert V, Law KS, Gottlieb CW, Bleicher SJ: Coated charcoal immunoassay of insulin. *J Clin Endocr* 25:1375–1384, 1965
20. Ho CK, Hashim SA: Pyridine nucleotide depletion in pancreatic islets associated with streptozotocin-induced diabetes. *Diabetes* 21:789–793, 1972
21. Yamamoto H, Uchigata Y, Okamoto H: Streptozotocin and alloxan induce DNA strand breaks and poly (ADP-ribose) synthetase in pancreatic islets. *Nature* 294:284–286, 1981
22. Okamoto H: Regulation of proinsulin synthesis in pancreatic islets, and a new aspect to insulin-dependent diabetes. *Mol Cell Biochem* 37:43–61, 1981
23. Masiello P, Bergamini E: Nicotinamide and streptozotocin diabetes in the rat: factors influencing the effectiveness of the protection. *Experientia* 33:1246–1247, 1977
24. Masiello P, Wollheim CB, Gori Z, Blondel B, Bergamini E: Streptozotocin-induced functioning islet cell tumor in the rat: high frequency of induction and biological properties of the tumor cells. *Toxicol Pathol* 12:274–280, 1984
25. Grill V, Westberg M, Östenson CG: B cell insensitivity in a rat model of non-insulin-dependent diabetes: evidence for a rapidly reversible effect of previous hyperglycemia. *J Clin Invest* 80:664–669, 1987
26. Grill V, Rundfeldt M: Abnormalities of insulin responses after ambient and previous exposure to glucose in streptozotocin-diabetic and dexamethasone-treated rats: role of hyperglycemia and increased B-cell demands. *Diabetes* 35:44–51, 1986
27. Malaisse WJ, Sener A, Koser M, Ravazzola M, Malaisse-Lagae F: The stimulus-secretion coupling of glucose-induced insulin release: insulin release due to glycogenolysis in glucose-deprived islets. *Biochem J* 164:447–454, 1977
28. Tancrède G, Rousseau-Migneron S, Nadeau A: Long-term changes in the diabetic state induced by different doses of streptozotocin in rats. *Br J Exp Pathol* 64:117–123, 1983
29. Blondel O, Bailbé D, Portha B: Relation of insulin deficiency to impaired insulin action in NIDDM adult rats given streptozotocin as neonates. *Diabetes* 38:610–617, 1989