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Metabolic Disturbances and Defects in Insulin Secretion in Rats With Streptozotocin-Nicotinamide-Induced Diabetes

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Summary

Rats with diabetes induced by streptozotocin (STZ) and nicotinamide (NA) are often used in animal studies concerning various aspects of diabetes. In this experimental model, the severity of diabetes is different depending on doses of STZ and NA. Moreover, diabetic changes in rats with STZ-NA-induced diabetes are not fully characterized. In our present study, metabolic changes and insulin secretion were investigated in rats with diabetes induced by administration of 60 mg of STZ and 90 mg of NA per kg body weight. Four to six weeks after diabetes induction, insulin, glucagon and some metabolic parameters were determined to evaluate the severity of diabetes. Moreover, insulin secretory capacity of pancreatic islets isolated from control and diabetic rats was compared. It was demonstrated that administration of 60 mg of STZ and 90 mg of NA per kg body weight induced relatively mild diabetes, since insulin, glucagon and other analyzed parameters were only slightly affected in diabetic rats compared with control animals. *In vitro* studies revealed that insulin secretory response was preserved in pancreatic islets of diabetic rats, however, was lower than in islets of control animals. This effect was observed in the presence of different stimuli. Insulin secretion induced by 6.7 and 16.7 mmol/l glucose was moderately reduced in islets of diabetic rats compared with control islets. In the presence of leucine with glutamine, insulin secretion appeared to be also decreased in islets of rats with STZ-NA-induced diabetes. Insulinotropic action of 6.7 mmol/l glucose with forskolin was also deteriorated in diabetic islets. Moreover, it was demonstrated that at a non-stimulatory glucose, pharmacological depolarization of plasma membrane with a concomitant activation of protein kinase C evoked significant rise in insulin release in islets of control and diabetic rats. However, in diabetic islets, this effect was attenuated. These results indicate that impairment in insulin secretion in pancreatic islets of rats with mild diabetes induced by STZ and NA results from both metabolic and nonmetabolic disturbances in these islets.

Key words

Rat • Diabetes • Streptozotocin • Nicotinamide • Pancreatic islets • Metabolism

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Introduction

Diabetes mellitus is a serious disease affecting about 5 % of people all over the world. Type 1 diabetes accounts for 5-10 % of all diabetic cases, whereas type 2 diabetes accounts for 90-95 % of cases. Type 1 diabetes is mainly due to autoimmune damage of pancreatic B-cells and patients are dependent on exogenous insulin. Type 2 diabetes develops more slowly, and the severity of metabolic disturbances is much lower. Blood glucose is only moderately elevated and peripheral insulin resistance leading to increased insulin secretion is usually observed. However, the compensatory hypersecretion of insulin leads to exhaustion of B-cells and to progressive B-cell failure. This type of diabetes is much more frequent in obese than in lean individuals. It is also well established that in diabetic patients various complications associated with diabetes develop over time (American Diabetes Association 2006).

Numerous efforts are continually being made to study diabetes and diabetes-related complications, to test new drugs and to develop new strategies in preventing and treating diabetes. Rodent models used in these studies differ significantly in relation to various aspects of diabetic abnormalities and therefore their utility is also differential (reviewed by Chatzigeorgiou *et al.* 2009, Masiello 2006, King 2012). In one of these models, diabetes is induced by administration of both streptozotocin (STZ) and nicotinamide (NA) to adult rats (Masiello *et al.* 1998). STZ is well known to cause damage of pancreatic B-cells (reviewed by Szkudelski 2001, Lenzen 2008), whereas NA partially protects these cells against the detrimental effects of STZ. In rats with this diabetic model, blood insulin may be slightly, moderately or deeply decreased depending on doses of STZ and NA. However, animals with diabetes induced by STZ and NA do not require exogenous insulin to survive (Masiello *et al.* 1998, Masiello 2006, Szkudelski 2012).

Rats treated with STZ and NA manifest symptoms of relatively mild diabetes compared with animals with diabetes induced by STZ alone. Importantly, B-cells in these rats are only partially damaged and therefore insulin secretion in response to glucose and some other stimuli is preserved. That is why this experimental model is often used in studies testing blood glucose-lowering properties and insulin-secretory properties of various pharmacological and natural compounds. This is also a suitable model to test the regenerative capacity of the endocrine pancreas. Results of many experiments demonstrated a real therapeutic value of different compounds in rats with diabetes induced by STZ and NA confirming the usefulness of this model in animal studies (for review see Masiello 2006, Szkudelski 2012). However, abnormalities in rats with STZ-NA-induced diabetes are not fully elucidated, particularly in relation to some metabolic defects and B-cell function. Our present study focuses on metabolic changes in rats with STZ-NA-induced diabetes and on insulin-secretory capacity of pancreatic islets derived from these animals. Characteristics of this experimental model will contribute to its better use in animal studies referring to different aspects of diabetes.

Methods

Animals, induction of diabetes and glucose tolerance test

Male Wistar rats purchased from Brwinow (Poland) were maintained in cages in an animal room with a 12:12-h dark-light cycle and a constant temperature of 21±1 °C. Animals had free access to tap water and were fed *ad libitum* a standard laboratory diet (Labofeed B, Kcynia, Poland). The experiments were performed according to rules accepted by Local Ethical Commission for Investigations on Animals.

Diabetes was induced by intraperitoneal administration of NA and STZ to rats weighing 160-180 g. Overnight fasted animals were administered NA (90 mg/kg) dissolved in 0.9 % NaCl, and after 15 min, STZ (60 mg/kg) dissolved in 0.1 mmol/l citrate buffer (pH 4.5) was injected.

It is known that blood glucose levels in STZ-NA-induced diabetic rats are different depending on experimental conditions (for review see Szkudelski 2012). Therefore, in our present study, glucose tolerance test was performed and rats with mild hyperglycemia were selected. After seven days, 20 control and 42 STZ-NA-treated rats were fasted overnight, blood samples were taken from the tail vein and glycemia was measured using a glucometer (HemoCue Glucose 201$^+$, Ängelholm, Sweden). Then, animals were given intragastrically 2 g glucose per kg body weight and glycemia was measured 30 and 60 min after glucose load.

In control rats, blood glucose measured 30 min after glucose administration, did not exceed 8 mmol/l, whereas in STZ-NA-treated animals, blood glucose levels differed substantially. In about 50 % of these rats, blood glucose was in the range of 12 to 15 mmol/l. These animals were classified as mildly diabetic and were selected for the experiments. In the remaining rats that were injected with STZ and NA, blood glucose measured 30 min after glucose administration was below 12 mmol/l (about 30 % of animals) or was higher than 15 mmol/l (about 20 %). These animals were excluded from the experiments. In the present study, mortality of STZ-NA-induced diabetic rats was less than 3 %.

Four to six weeks after induction of diabetes, 20 non-diabetic and 20 diabetic rats was killed by decapitation and blood serum and liver samples were taken and stored (−20 °C) until analysis. Moreover, 9 non-diabetic and 9 diabetic rats was taken from among these animals and used in insulin secretion studies.

Determinations of insulin, glucagon and metabolic parameters

Concentrations of insulin and glucagon were measured radioimmunologically using kits specific for rat hormones. Blood glucose was determined by the

enzymatic method with glucose oxidase, peroxidase and dianisidine (Bergmeyer and Bernt 1974), blood free fatty acids and triacylglycerols were determined colorimetrically by the method of Duncombe (1964) and Foster and Dunn (1973), respectively. Lactate was determined by the measure of NADH formed from NAD^+ in the presence of lactate dehydrogenase (Everse 1975). Liver triacylglycerols were extracted (Folch *et al.* 1975) and determined as blood triacylglycerols. Liver glycogen was hydrolyzed with amyloglucosidase as described previously (Szkudelski 2005) and determined as blood glucose.

Determinations of TNF-α and IL-1β

Tumor necrosis factor alpha (TNF-α) and interleukin-1 beta (IL-1β) levels in blood serum of control and diabetic rats were determined by ELISA kits. Analyses were performed according to the instructions provided by the manufacturer using diluted and nondiluted blood samples. The optical density of samples was read using a microplate reader (Synergy 2, BioTek). According to the information of the manufacturer, the minimum detectable doses of rat TNF-α and IL-1β using this method are less than 5 pg/ml.

Isolation of pancreatic islets

Pancreatic islets of control and diabetic rats were isolated by collagenase digestion as described previously (Lacy and Kostianovsky 1967, Szkudelski 2006, Zywert *et al.* 2011). Hanks' solution saturated with a mixture of O_2/CO_2 (95 %/5 %) was used during isolation. The composition of the solution was the following (in mmol/l): NaCl 137, KCl 5.36, $MgSO_4$ 0.81, Na_2HPO_4 0.34, KH_2PO_4 0.44, $CaCl_2$ 1.26, $NaHCO_3$ 4.17. At the beginning of isolation, Hanks' solution was injected into the common bile duct and the pancreas was excised. In each experiment, glands pooled from three rats were cut down with scissors and incubated with collagenase. After digestion, islets were washed with Hanks' solution without the enzyme and were separated from the remaining exocrine tissue by hand picking under a stereomicroscope.

Insulin secretion from pancreatic islets

In vitro studies were performed to compare the insulin-secretory response of pancreatic islets isolated from non-diabetic and diabetic rats. Islets derived from both groups of animals were incubated for 90 min in Krebs-Ringer buffer, pH 7.4. The composition of the buffer was (in mmol/l): 115 NaCl, 24 $NaHCO_3$, 5 KCl, 1 $MgCl_2$, 1 $CaCl_2$ and 0.5 % bovine serum albumin. Pancreatic islets were incubated in an atmosphere of O_2/CO_2 (95 %/5 %) at 37 °C with gentle shaking. Basal insulin release from islets of control and diabetic rats was studied in the presence of 2.8 mmol/l glucose. To compare insulin secretion induced by stimulatory glucose concentrations, islets of non-diabetic and diabetic animals were exposed to 6.7 or 16.7 mmol/l glucose. Apart from stimulation by glucose, the insulinotropic effects of leucine and glutamine were studied in islets of control and diabetic rats. In these experiments, pancreatic islets derived from both groups of animals were exposed to 10 mmol/l leucine with 10 mmol/l glutamine. Moreover, islets of control and diabetic rats were stimulated by 6.7 mmol/l glucose in the presence of 1 μmol/l forskolin. Additionally, pancreatic islets were incubated in the presence of 2.8 mmol/l glucose, 5 μmol/l glibenclamide and 100 nmol/l PMA and insulin release was compared between control and diabetic islets.

At the end of each incubation, samples of Krebs-Ringer buffer were stored (−20 °C) until insulin determination.

MTT assay

MTT assay was performed according to the method described by Janjic and Wollheim (1992). Pancreatic islets isolated from control and diabetic rats were preincubated at 37 °C in Krebs-Ringer buffer containing 6.7 mmol/l glucose. Then, islets were incubated at 37 °C in the presence of 0.5 mg/ml MTT. After this incubation, islets were incubated with isopropanol at room temperature. Each incubation was performed for 90 min. After the final incubation, isopropanol absorbance was read at 560 nm.

Reagents

Streptozotocin, nicotinamide, D-glucose, L-leucine, L-glutamine, forskolin, glibenclamide, bovine serum albumin (fatty acid free), dimethyl sulfoxide (DMSO), thiazolyl blue tetrazolium bromide (MTT), phorbol 12-myristate 13-acetate (PMA) and reagents used to prepare Krebs-Ringer buffer and Hanks' solution and to determine metabolic parameters in rat tissues were obtained from Sigma (St. Louis, USA). Collagenase P was from Roche Diagnostics GmbH (Mannheim, Germany). Kits used to determine insulin and glucagon were from Millipore Corp. (St. Charles, USA), whereas TNF-α and IL-1β kits were from R&D Systems Inc. (Minneapolis, USA).

Stock solutions of forskolin, glibenclamide and PMA were prepared in DMSO. The final concentration of the solvent in Krebs-Ringer buffer with islets did not

exceed 0.1 %.

Statistical analysis

In the *in vivo* experiments, means ± SEM were calculated for 20 non-diabetic and 20 diabetic rats. In the case of insulin secretion studies, means ± SEM were calculated for 12 batches of islets from three separate experiments. All data were evaluated statistically using Student's t-test. Differences were considered statistically significant at $p<0.05$.

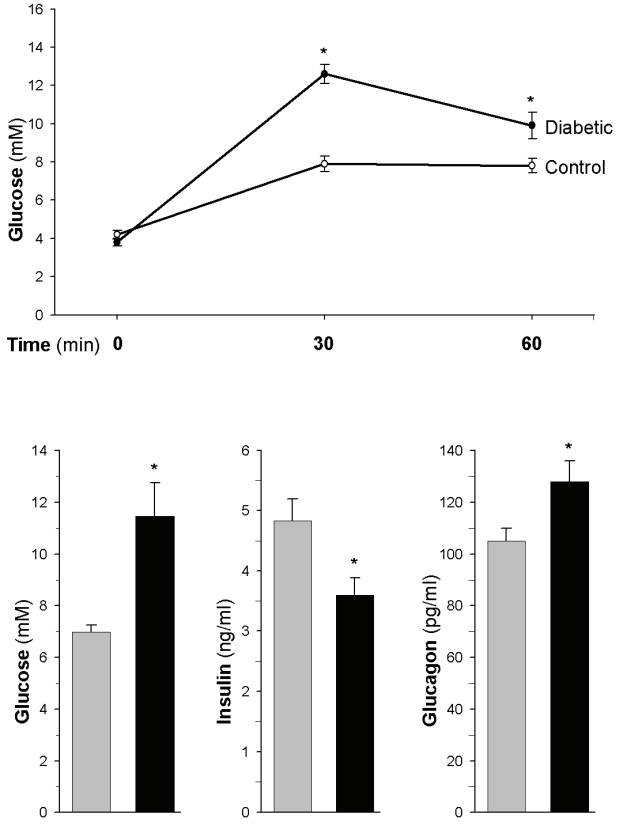

Fig. 1. Fasting blood glucose before and after glucose load measured one week after induction of diabetes (top) and non-fasting blood glucose, insulin and glucagon levels four to six weeks after induction of diabetes (bottom) in control (grey bars) and diabetic (black bars) rats. Values represent means ± SEM for 20 animals. * significant difference ($p<0.05$) between control and diabetic rats

Results

Glucose tolerance test, insulin, glucagon and metabolic parameters

In the present study, numerous differences in analyzed parameters between control rats and rats with STZ-NA-induced diabetes rats were shown. Although fasting blood glucose levels, determined seven days after induction of diabetes, were similar in control and diabetic rats, glucose tolerance test revealed differences in glycemia between both groups of animals. It was demonstrated that in diabetic rats, blood glucose was significantly higher 30 and 60 min after intragastric glucose load compared with glycemia in control rats (Fig. 1).

Blood insulin, glucagon and some metabolic parameters determined four to six weeks after induction of diabetes were also different in control and diabetic rats. Blood insulin was found to be decreased by 25 % in diabetic rats, whereas blood glucagon was increased in these rats by 20 % compared with control animals. Non-fasting blood glucose in diabetic rats was increased by 65 % compared with control animals (Fig. 1).

It was also demonstrated that concentrations of blood triacylglycerols and free fatty acids were mildly increased (by 40 % and 25 %, respectively) in rats with diabetes compared with control animals. Blood lactate was increased by 18 % in diabetic rats. Liver triacylglycerol accumulation was found to be increased by 50 % in rats with experimental diabetes, whereas liver glycogen content was unchanged (Table 1).

TNF-α and IL-1β

Concentrations of TNF-α and IL-1β in blood of control rats and rats with STZ-NA-induced diabetes were below the detectable levels (Table 1).

Insulin secretion from pancreatic islets

Exposure of pancreatic islets isolated from control and diabetic rats to physiological and supraphysiological glucose concentrations induced a clear-cut, concentration-dependent, rise in insulin secretion compared with basal secretion measured in the presence of 2.8 mmol/l (Fig. 2). These results indicate that pancreatic islets isolated from both groups of animals were metabolically active and effectively responded to stimulation by glucose. Experiments on pancreatic islets derived from control rats and rats with STZ-NA-induced diabetes revealed, however, substantial differences in the insulin-secretory response between both groups of islets. Although basal insulin output, measured at 2.8 mmol/l glucose, did not differ significantly, insulin secretion induced by 6.7 mmol/l glucose appeared to be diminished by 20 % in islets of diabetic rats compared with control islets. Similarly, the insulin-secretory response to 16.7 mmol/l glucose was significantly impaired in islets of diabetic animals since these islets released 17 % less insulin than islets of control rats (Fig. 2).

Table 1. Metabolic parameters and proinflammatory cytokines in control and diabetic rats.

Parameter		Control rats	Diabetic rats
Blood	*Triacylglycerols (mmol/l)*	3.633 ± 0.169	5.200 ± 0.274*
	Free fatty acids (mmol/l)	0.217 ± 0.014	0.272 ± 0.017*
	Lactate (mmol/l)	5.634 ± 0.165	6.696 ± 0.180*
	TNF-α and IL-1β	Undetected	Undetected
Liver	*Triacylglycerols (mg/g)*	9.385 ± 0.956	19.972 ± 3.221*
	Glycogen (mg/g)	60.060 ± 8.230	58.152 ± 9.985

Values represent means ± SEM (n=20). Diabetic rats represent a group of streptozotocin-nicotinamide-treated animals with relatively mild diabetes (see Methods). * significant difference ($p<0.05$) between control and diabetic rats

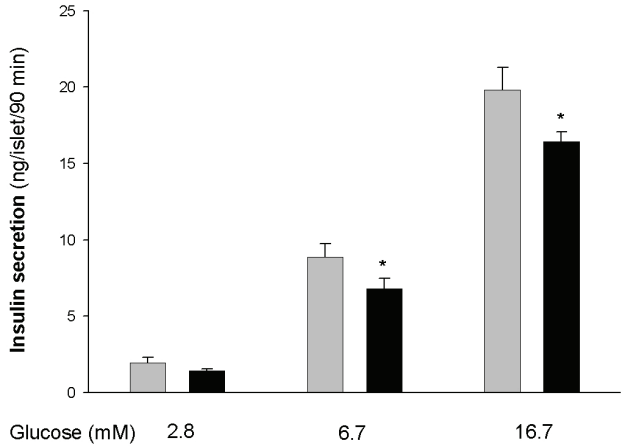

Fig. 2. Insulin secretion induced by glucose from pancreatic islets of control (grey bars) and diabetic (black bars) rats. Values represent means ± SEM of 12 determinations from 3 separate experiments. * significant difference ($p<0.05$) between control and diabetic rats

It was also demonstrated that insulin secretory response to 6.7 mmol/l glucose and 1 μmol/l forskolin was reduced in pancreatic islets of rats with STZ-NA-induced diabetes. Islets of these animals released 40 % less insulin compared with islets of non-diabetic rats (Fig. 3). The insulinotropic action of 10 mmol/l leucine in the combination with 10 mmol/l glutamine was also significantly affected in pancreatic islets derived from diabetic rats. Mean insulin secretion elicited by these amino acids was diminished by 20 % in diabetic islets (Fig. 3). It was also demonstrated that insulin release induced by 5 μmol/l glibenclamide and 100 nmol/l PMA in the presence of 2.8 mmol/l glucose was impaired in diabetic islets. Under these conditions, islets of diabetic rats released 40 % less insulin that islets of control animals (Fig. 3).

MTT assay

Incubations of pancreatic islets with MTT demonstrated that reduction of this compound to formazan differed in islet cells of control and diabetic rats. Formazan formation was decreased in islets of diabetic rats by about 15 % compared with islets of control rats (data not shown).

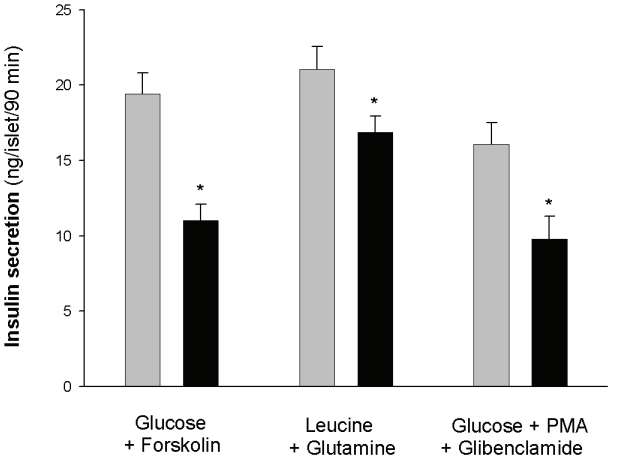

Fig. 3. Insulin secretion induced by 6.7 mmol/l glucose and 1 μmol/l forskolin (left), 10 mmol/l leucine with 10 mmol/l glutamine (middle) or 2.8 mmol/l glucose, 5 μmol/l glibenclamide and 100 nmol/l phorbol 12-myristate 13-acetate (PMA, right) from islets of control (grey bars) and diabetic (black bars) rats. Values represent means ± SEM of 12 determinations from 3 separate experiments. * significant difference ($p<0.05$) between control and diabetic rats

Discussion

The severity of pathological changes in animals with diabetes induced by STZ and NA differs depending on experimental conditions. In our present study, pancreatic hormones and metabolic parameters were determined to evaluate the severity of experimentally induced diabetes. It was demonstrated that fasted glycemia was similar in control and diabetic rats, but after glucose load, blood glucose was slightly higher in

diabetic rats. It was also found that insulin, glucagon and metabolic parameters were only slightly affected by diabetes. Moreover, in rats with STZ-NA-induced diabetes, similarly to control animals, blood levels of proinflammatory cytokines, TNF-α and IL-1β, remained below detectable values. This is in contrast to rats with diabetes induced by STZ alone since in these animals blood levels of TNF-α and IL-1β were reported to be increased (Jain *et al.* 2006, Olukman *et al.* 2010). All these results indicate that rats with STZ-NA-induced diabetes used in the present study manifested symptoms of relatively mild diabetes compared with STZ-induced diabetic rats.

Partial damage of pancreatic B-cells is pivotal in rats with STZ-NA-induced diabetes since reduced glucose tolerance in these animals is thought to result from decreased insulin secretion and not from peripheral insulin resistance (Masiello *et al.* 1998, Masiello 2006, Szkudelski 2012). However, metabolic and functional defects in B-cells of rats with STZ-NA-induced diabetes are not fully elucidated. It is known that STZ alone induces necrosis of B-cells, whereas administration of both STZ and NA causes only partial destruction of these cells (Masiello *et al.* 1998, Szkudelski 2001, 2012, Lenzen 2008). Islets of rats with STZ-NA-induced diabetes are characterized by both reduced B-cell mass and metabolic abnormalities in the insulin-secreting cells. *In vitro* studies demonstrated that glucose-induced insulin secretion is usually diminished, especially at higher concentrations of the sugar, and the second phase of insulin secretion is lacking (Masiello *et al.* 1998, Novelli *et al.* 2001, Fierabracci *et al.* 2002, Masiello 2006, Szkudelski 2012). In our present study, insulin secretion stimulated by both physiological and supraphysiological glucose appeared to be decreased by about 20 % in islets of diabetic rats compared with normal islets. This decrease is relatively low since in rats that were given STZ alone, insulin release induced by glucose is known to be almost completely suppressed (Masiello *et al.* 1998).

The impairment in glucose-induced insulin release in rats with STZ-NA-induced diabetes is thought to result predominantly from reduced activity of FAD-glycerophosphate dehydrogenase, the key enzyme of the glycerophosphate shuttle in B-cells (Novelli *et al.* 2001). However, other metabolic defects cannot be excluded. Indeed, in our present study, formazan production from MTT, reflecting metabolic activity of islet cells (Janjic and Wollheim 1992), was demonstrated to be reduced in islets of diabetic rats.

However, despite evidence of metabolic changes in B-cells leading to decrease in glucose-induced insulin secretion, previous studies have reported that insulin release induced by α-ketoisocaproate (an insulin secretagogue metabolized exclusively in mitochondria) or by sulfonylurea tolbutamide is similar in B-cells of control and diabetic rats (Masiello *et al.* 1998, Novelli *et al.* 2001). Moreover, conversely to STZ-diabetic rats (Okabayashi *et al.* 1989), insulin-secretory response to arginine was found to be exaggerated in perfused pancreas and isolated islets of rats with STZ-NA-induced diabetes (Masiello *et al.* 1998, Novelli *et al.* 2004). In our present study, the insulinotropic activity of leucine with glutamine was determined in islets of control and diabetic rats to compare insulin secretion induced by metabolizable stimuli other than glucose. The combination of these amino acids has a potent insulin secretory effect, however, the mechanism of this action is different from that of glucose and arginine. In B-cells, leucine is a mitochondrial fuel, but acts also acts as an allosteric activator of glutamate dehydrogenase and thereby enhances glutaminolysis (Fahien and MacDonald 2011). Exposure of islets isolated from control and diabetic rats to leucine with glutamine significantly enhanced insulin release, however, in diabetic islets the insulinotropic effect was attenuated. This suggests that defects related with mitochondrial metabolism of these amino acids may be present in B-cells of rats with STZ-NA-induced diabetes.

Glucose-induced insulin secretion is known to be significantly potentiated by factors increasing cAMP levels in B-cells. Under physiological conditions, predominant role in this action is ascribed to gut-derived incretin hormones (reviewed by Holst and Gromada 2004). In our present study, exposure of pancreatic islets to glucose in the combination with forskolin, to activate protein kinase A and, thereby, to increase cAMP levels, resulted in a substantial increase in insulin secretion in control and diabetic islets. The insulinotropic action of glucose and forskolin was, however, markedly deteriorated in islets of rats with STZ-NA-induced diabetes. This indicates that the diminished ability of glucose to stimulate insulin secretion from diabetic islets is not compensated by cAMP-generating agents. It was also demonstrated that in the presence of glucose and forskolin, the difference in the insulin-secretory response between control and diabetic islets was higher than in the presence of glucose alone. These results suggest that B-

cells of diabetic rats are less sensitive to factors potentiating insulin secretion *via* cAMP. This effect may result from disturbed generation and/or action of cAMP in B-cells of rats with STZ-NA-induced diabetes. Defective cAMP-secretion coupling was also recently described in pancreatic islets of the mildly diabetic Goto-Kakizaki rat, a rodent model of the nonobese type 2 diabetes (Dolz *et al.* 2011).

Literature data indicate that decrease in insulin secretion in rats with STZ-NA-induced diabetes is predominantly due to metabolic defects in B-cells. However, other abnormalities, not directly related with metabolism of glucose and some other secretagogues, may be present in B-cells and contribute to reduced insulin secretion. Therefore, in the further part of our study, pancreatic islets of control and diabetic rats were compared when insulin release was induced without metabolic events. In these experiments, islets were incubated in the presence of low glucose, and insulin release was induced *via* pharmacological depolarization of plasma membrane and simultaneous activation of protein kinase C. Under these conditions, some compounds reducing insulin secretion *via* inhibition of glucose metabolism in B-cells were previously demonstrated to be ineffective (Szkudelski 2007). Incubations of pancreatic islets with glibenclamide, to depolarize plasma membrane, and with PMA, to activate protein kinase C, significantly enhanced insulin release despite a non-stimulatory glucose concentration. A clear-cut increase in hormone secretion was found in both control and diabetic islets, however, in the latter, insulin secretory response was markedly diminished compared with control islets. This indicates that decreased ability of diabetic islets to secrete insulin in response to glucose and some other secretagogues is not only due to metabolic defects in B-cells, but other abnormalities are present in these islets and contribute to reduced secretion of insulin.

In conclusion, our present study demonstrated that administration of the appropriate doses of STZ and NA to adult rats induces relatively mild diabetes. Pancreatic islets of these rats release less insulin in response to physiological and supraphysiological glucose compared with islets of control animals. However, the insulin secretory response to glucose is only slightly diminished. It was also observed that impairment in glucose-induced insulin secretion in islets of STZ-NA-induced diabetic rats is not ameliorated by increase in cAMP levels in islet cells. The insulinotropic action of secretagogues other than glucose, i.e. leucine with glutamine, was found to be also decreased in diabetic islets. Moreover, the present study demonstrated that, apart from metabolic defects in B-cells, other abnormalities are present in pancreatic islets of STZ-NA-induced diabetic rats and contribute to diminished secretion of insulin.

The experimental model of STZ-NA-induced diabetes has some similarities with type 2 diabetes in humans with reduced B-cell mass (e.g. partially reduced insulin secretion in response to glucose and glucose with cAMP-generating agents). Therefore, this diabetic model may be very useful in studies related with different aspects of type 2 diabetes in humans.

Conflict of Interest

There is no conflict of interest.

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