Serum amylase activity disorders in the course of experimental diabetes in rabbits

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ABSTRACT: We evaluated the influence of experimental diabetes on the amylase release in exocrine pancreatic cells. Following a single alloxan injection, male rabbits were divided into control and four experimental groups according to the disease duration. Respectively after 21, 42, 90, and 180 days intervals, the final levels of glucose and amylase in the sera were determined spectrophotometrically by the use of an enzymatic method. The mean serum glucose level in healthy controls was 6.4 mmol/l. It reached its top value of 32.0 mmol/l after 42 days of diabetes, and decreased to 23.12 mmol/l on day 180. The mean serum amylase activity was 124 U/l in healthy controls, 57 U/l on day 21, 138 on day 42, 84 U/l on day 90, and 56 U/l after 180 days of diabetes. The initial drop of the amylase activity may be interpreted as a result of decreased stimulating insulin effect on exocrine pancreatic cells, whereas the transient increase in activity on day 90 seems to be related to the increased destruction processes of the pancreatic tissue.

Keywords: amylase; experimental diabetes; pancreatic exocrine activity; rabbits

Exocrine pancreatic functions in the course of diabetes have been a subject of numerous studies in recent years, especially in patients with insulin-dependent diabetes mellitus (Hardt et al., 2000; Lernmark, 2000). Clinical investigations and experimental laboratory research supported by morphological and ultrastructural observations indicate a close relation between pancreatic exo- and endocrine parts (Okabayashi et al., 1988; Aughsteen and Kataoka, 1993; Meral et al., 2001) Insulin and glucagon actions have been investigated in great detail and clinical studies in diabetic patients show that the impairment of pancreatic exocrine functions is due to insulin deficiency and relative glucagon predominance (Adeghate, 1999; Taniyama et al., 1999; Hardt et al., 2000; Lernmark, 2000; Harding et al., 2001). Reports regarding the influence of insulin deficiency on the pancreatic exocrine function describe distinctive changes in composition and quality of pancreatic juice as well as its decreased production (Okabayashi et al., 1988; Aughsteen and Kataoka, 1993; Kim et al., 2000). Interestingly, the relation between insulin and glucagon concentrations in extracellular fluid and their antagonistic actions also seem to play a key role in pancreatic exocrine function disorders. Insulin is recognised as the main factor stimulating exocrine activity, whilst glucagon is ascribed an inhibitory role, not only in the processes of synthesis but also in the secretion of amylase and HCO_3^- ions (Taniyama et al., 1999; Hardt et al., 2000), as shown in both functional tests as decreased enzyme activity and ionic shifts, and in histopathological observations in the form of involutional changes, exocrine cell degranulation and texture atrophy (Minami et al., 1999; Harding et al., 2001). Amylase – one of the main enzymes produced in exocrine pancreatic cells, may be recognised as an adequate indicator of organ's activity both in physiological and pathological states. The aim of the present study was to evaluate the influence of experimental diabetes on the amylase release and following serum concentrations.

MATERIAL AND METHODS

The project protocol was fully approved by the Bioethical Committee and the study was conducted

according to the guidelines of good animal practice (Maciejewski et al., 1999a,b, 2001).

Two hundred male rabbits, New Zealand breed, weighing 2.75–3.30 kg were used in the experiment. Animals were housed one per cage under a 12 h/12 h light/dark cycle at 21 ± 2°C temperature and 50% relative humidity with standard granulated food and water available. Mortality rate in the whole course of experiment was 55.5%. Diabetes mellitus was induced by a single injection of alloxan (Sigma Chemical Company, St. Louis, MO, USA) at a dose of 10 mg/kg into the auricular vein (Goi et al., 1987). On day 7 blood glucose levels were measured with glucometer (Boehringer, Germany) to confirm the presence of diabetes (glucose level > 11.1 mmol/l). The onset of diabetes was counted up from this point in time, and glucose levels measured in weekly intervals. The rabbits which survived were divided into the following groups: Group 1 – controls (n = 18), Group 2 – 21 days diabetes (n = 18), Group 3 – 42 days diabetes (n = 17), Group 4 – 90 days diabetes (n = 19), Group 5 – 180 days diabetes (n = 17). After the above-mentioned periods blood samples were taken and the rabbits were anaesthetized and decapitated. The final levels of glucose in the sera were determined spectrophotometrically by an enzymatic method using ready kit GS-120L (Cormay, Lublin, Poland) at wavelength 500 nm. Blood serum α-amylase activity was determined by an enzymatic colorimetric test using 2-choloro-4-nitrofenylo-α-maltrioside (CNP-G3) (Kurahashi and Inomata, 1988).

Statistical analysis. The statistical analysis was done using the SAS system v. 6.11. Results are expressed as means ± SD. Differences between groups were analysed by one-way ANOVA, and if significant paired t-test was used between individual data points. P-values are two-sided and considered significant when $P < 0.05$.

RESULTS

Body weight changes. During the whole experiment average body weight of rabbits in the control group remained almost unchanged, with the mean level of 2 904 ± 9 grams. The average body weight values in particular experimental groups were as follows: Group 2 – 3 118 ± 14 grams (initial) and 2 967 ± 32 grams (final). Group 3 – 2 607 ± 3 (initial) and 2 541 ± 23 grams (final). Group 4 – 3 009 ± 45 (initial) and 2 913 ± 14 grams (final). Group 5 – 2 503 ± 7 (initial) and 2 398 ± 2 grams (final). No significant changes were found between the control and the experimental groups.

Serum glucose levels. The initial serum glucose concentration was 6.4 ± 2.0 mmol/l (Group 1). 21 days after injection of alloxan, the concentration increased to 21.8 ± 9.5 mmol/l (Group 2). It reached the peak level 32.0 ± 19.1 mmol/l on day 42 (Group 3), and decreased through 29.1 ± 11.2 mmol/l on day 90 (Group 4), and finally dropped to 23.2 ± 10.9 mmol/l on day 180 (Group 5). All differences were statistically significant compared to baseline ($P < 0.05$), with glucose concentrations being expressed in the graphic form in Figure 1.

Serum α-amylase activities. The mean initial serum α-amylase activity in healthy animals (Group 1) was 124 ± 30 U/l. On day 21 of the disease (Group 2) it dropped to the mean value of 57 ± 24, which was 46% of the level of healthy controls ($P < 0.05$). In the animals with 42-day diabetes (Group 3), an

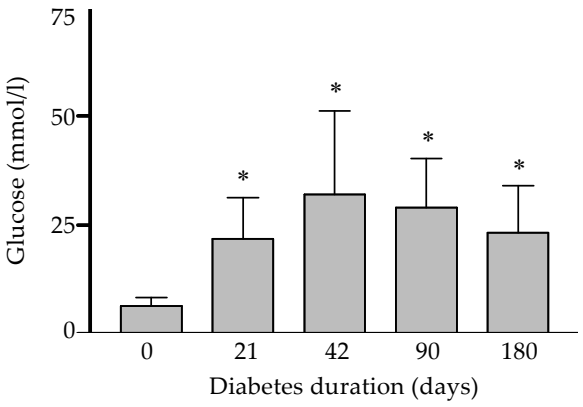

Figure 1. Serum glucose concentrations in relation to disease duration in the course of experimental diabetes (*$P < 0.05$)

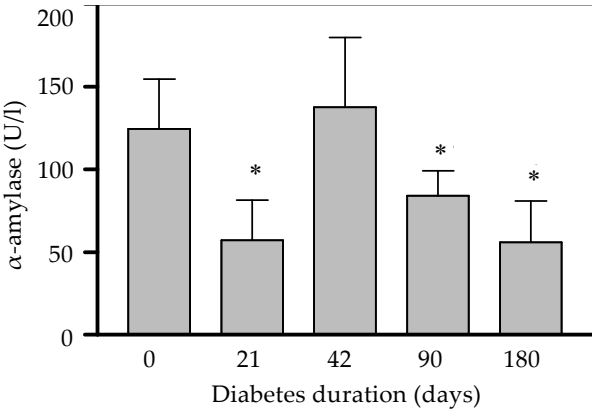

Figure 2. Serum amylase concentrations in relation to disease duration in the course of experimental diabetes (*$P < 0.05$)

increase in the enzyme activity up to 138 ± 42 U/l was observed, which reflected 111% of the initial value. Following the course of the disease, a gradual decrease was noted, reaching 84 ± 15 (68% of its initial value in Group 4) on day 90 ($P < 0.05$). After 180 days of diabetes (Group 5), the lowest mean enzyme activity of 56 ± 25 U/l, that is 45% of the value in the healthy controls, was noted ($P < 0.05$). The above results are illustrated in Figure 2.

DISCUSSION

The current study is a continuation of a larger project, with partial results having been published previously (Maciejewski et al., 1999a,b, 2001). Choosing serum α-amylase as an indicator of the exocrine pancreatic function is based on the enzyme's relative stability, long breakdown period, high sensitivity and specificity, in comparison with other pancreatic enzymes being also used in daily clinical practice.

The impaired exocrine pancreatic function in diabetic patients may be a result of multiple factors, the most important being glucagon inhibiting influence and lack of insulin stimulation on synthesis in exocrine cells (Hardt et al., 2000; Harding et al., 2001). Lack or attenuation of neurovegetative stimulation following diabetic neuropathy also leads to decreased enzyme secretion (Boiadzhieva, 1990). Moreover, capillary lesions as a result of diabetic microangiopathy impair diffusion of factors that can stimulate synthesis and output of pancreatic juice ingredients (Goi et al., 1987). While examining an alloxan-induced model of diabetes, direct inhibiting influence of this substance on adenylate cyclase should also be considered, which through a decrease in cAMP level impairs extrareceptor mechanisms in the exocrine cell area (Shimizu et al., 2000). Changes in pancreatic enzyme composition have been described by various research teams, while most authors report a significant drop in protein concentrations, reaching 30–50% of the values measured in healthy controls. Focusing on the α-amylase activity in particular, an even more significant drop down to the level of 20–30% of that in the control groups was observed (Alvarez and Lopez, 1989; Hegyi et al., 1999). Interestingly, insulin administration to the animals had some normalising effects by increasing the pancreatic juice proteins levels in the group of animals with fully developed diabetes. This however was not true of α-amylase as its activity tended to remain decreased (Ahren and Sundkvist, 1995).

Present measurements of α-amylase activity in the rabbit serum have shown a characteristic distribution. Within the first three weeks of diabetes, the mean enzyme activity dropped to the levels below the half of the values noted in healthy controls. In the following three-week period the mean α-amylase activity increased to the levels similar to the control group, followed by a persistent decline throughout the study. Reports in the available literature regarding these phenomena are inconsistent (Hirano et al., 1992; Zhang and Roomans, 1999). It seems that the initial drop in the serum α-amylase activity may be interpreted by the impaired pancreatic exocrine secretion due to a decrease in the insulin stimulatory action (Aughsteen and Kataoka, 1993; Hegyi et al., 1999; Hardt et al., 2000; Shimizu et al., 2000). Transient increase in enzyme activity on day 90 of the experiment may reflect the release of the enzyme from cellular compartments caused by increased destructive processes, which is consistent with observations on the ultrastructural level (Ahren and Sundkvist, 1995; Maciejewski et al., 1999a,b, 2001). The following decrease in enzyme activity is a result of definite depletion of α-amylase reserves as well as its impaired synthesis in the exocrine cells.

The present study confirms the existence of a close functional relation between pancreatic endo- and exocrine parts, the latter being measured by serum amylase activity, in the course of alloxan-induced experimental diabetes. The characteristic pattern of the measured enzyme activities during the course of experimental diabetes reflects the dynamics and chronology of processes present in the exocrine pancreatic cells. The observed systemic and metabolic disorders were accompanied by relevant transformations on the ultrastructural level. The number and intensity of these changes were proportional to the disease duration as described by the authors in their previous studies (Maciejewski et al., 1999a,b, 2001).

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