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Diabetes mellitus alters lysosomal enzymes activity in rabbit epididymis

Cukrzycy zaburza aktywność enzymów lizosomalnych najądrza królika

Diabetes is a metabolic disease caused by inappropriate insulin secretion and/or function. It could be complicated by abnormalities of various organs, including nephropathy, retinopathy and others. The primary disorder may also affect reproductive function and alter levels of sex hormones [1, 2, 7, 10–13, 21]. In men suffering from diabetes, functional disturbances of ejaculation (i.e., reverse ejaculation into the urinary bladder), as well as calcinosis of the deferent ducts were also reported [8]. Moreover, Soudamani et al. [19] revealed decrease of rat epididymal weight in the experimentally induced diabetes. The mechanisms leading to such complications are complex. Our previous data suggest that infertility [28] and some diabetic complications could be the consequence of abnormal activity of lysosomal enzymes [14–17, 21–27].

The aim of the current study was to evaluate the activity of the free and bound fraction of selected lysosomal enzymes in rabbits with the experimentally induced diabetes mellitus.

MATERIAL AND METHODS

The study was performed according to the general principles for the animal experimentation and approved by the Bioethical Committee of the Medical University of Lublin. Mature, New Zealand male rabbits (Chorzelów, Poland) were housed in proper stainless steel cages under standard laboratory conditions. Laboratory and filtrated tap water were provided *ad libitum.*

The experiment was described in details elsewhere [14–16, 21–27]. Diabetes mellitus was developed by the alloxan (Sigma Chemical Co., St. Louis, USA) intravenous administration to the marginal auricular vein. A week later, the glucose level was measured in the sera by the automatic enzymatic method (Dextrostix; BAYER, Austria). The level >11.1 mmol/l (>200 mg%) indicated diabetes mellitus and the animals were randomly selected to four experimental groups (Tables 1 and 2). The experimental day 7 was regarded as the first day of the disease. Rabbits were sacrificed by the spinal cord dislocation, on days 21, 42 and 180, respectively. According to the principles of the Bioethical Committee, only one untreated control group was designated and the animals from this group were sacrificed on the last day of the experiment.

During the autopsy the right epididymis was removed, weighed, frozen in liquid nitrogen

and stored at -20°C until biochemical studies. After being defrosted, the body of each organ was homogenized and the activity of the bound and free fraction of acid phosphatase, beta-galactosidase, beta-N-acetyl-glucosaminidase, cathepsin B, D and L, lipase, and sulphatase were determined spectrophotometrically (Specol 21 – Carl Zeiss; Jena, Germany) by methods described in detail previously [4]. All the reagents were obtained from Sigma Chemical Co (St. Luise, USA).

The data was statistically evaluated using STATISTICA 5.0 software. The type of distribution was examined using Kolmogoroff-Smirnoff test. Due to normal distribution and homogeneity of variance the data were analyzed by ANOVA followed by Duncan test. $\alpha=0.05$ ($p<0.05$) was considered significant.

RESULTS

On day 21 the activity of the bound fraction of acid phosphatase was significantly decreased, while beta-galactosidase and all the examined cathepsins were increased (Table 1). Simultaneously, an increased activity of the free fraction of cathepsin D and a decrease of beta- galactosidase were revealed (Table 2).

Table 1. The bound fraction activity (Mean±SD) of acid phosphatase, beta-galactosidase, beta-N-acetyl-glucosaminidase, lipase, (pmol/mg of protein/hr), cathepsin B, D and L, and sulphatase (nmol/mg of protein/hr) in rabbit epididymis

	Control	Day of diabetes			
		21	42	90	180
Acid phosphatase	235.72±30.96	104.05±13.45*	257.26±60.22	109.54±22.77*	198.72±44.50
Beta-galactosidase	4.43±1.20	5.17±1.01*	7.23±2.53*	3.24±0.61	8.22±3.46*
Beta-*N*-acetyl-glucosaminidase	31.76±7.82	33.93±4.76	40.11±19.43	40.65±11.92	34.11±24.25
Cathepsin B	8.03±2.21	11.09±2.45*	8.37±4.85*	7.02±2.35	6.94±3.90
Cathepsin D	92.30±13.72	269.35±36.70*	89.93±15.29	189.90±53.01*	179.64±70.82*
Cathepsin L	32.07±5.32	129.42±9.17*	43.64±16.39	42.90±22.92	64.58±30.64
Lipase	33.40±11.58	31.99±8.52	46.60±14.89	46.97±10.26	33.08±10.10
Sulphatase	1.51±0.27	1.53±0.29	1.50±0.43	1.50±0.54	0.45±0.27

*p<0.005 vs control

Table 2. The free fraction activity (Mean±SD) of acid phosphatase, beta-galactosidase, beta-*N*-acetyl-glucosaminidase, lipase, (pmol/mg of protein/hr), cathepsin B, D and L, and sulphatase (nmol/mg of protein/hr) in rabbit epididymis

	Control	Day of diabetes			
		21	42	90	180
Acid phosphatase	345.26±32.45	262.81±41.94	240.30±41.22	171.28±46.29*	227.6±45.42
Beta-galactosidase	3.84±1.21	3.49±1.25*	5.63±2.03	2.07±0.76	4.66±1.76
Beta-*N*-acetyl-glucosaminidase	44.58±9.80	40.00±6.15	48.68±17.91	30.83±7.66*	36.53±9.13*
Cathepsin B	9.40±3.99	9.15±2.95	8.66±2.57	8.46±3.19	11.16±7.124
Cathepsin D	157.57±28.33	279.25±68.01*	167.85±41.72	194.0±45.66	197.08±74.85
Cathepsin L	40.71±9.34	38.05±16.01	25.49±13.30	46.42±12.25*	60.78±37.14
Lipase	14.46±2.66	15.57±4.19	13.75±3.47	18.11±3.04	13.36±3.37
Sulphatase	0.32±0.13	0.11±0.01	0.30±0.05	0.27±0.12	0.28±0.12

*p<0.005 vs control

A statistically significant increase of the bound fraction of beta-galactosidase and cathepsin D was found on day 42. Lack of significant changes was observed in the free fraction of all the evaluated lysosomal enzymes. On day 90 the activity of both fractions of acid phosphatase was decreased. Moreover, an increase of the bound fraction of cathepsin D and the free fraction of cathepsin L were revealed. The activity of the beta-*N*-acetyl-glucosaminidase free fraction was significantly decreased. The bound fraction of beta-galactosidase and cathepsin D was significantly increased on day 180. On the same day, the activity of the free fraction of beta-*N*-acetyl-glucosaminidase was decreased. No significant differences were found for lipase and sulphatase throughout the whole experiment (Table 1 and 2).

DISCUSSION

The main function of epididymal cells, i.e., principal, narrow, clear and basal ones, is endocystosis, as well as synthesis and secretion of various factors that keep the proper local environment for the sperm maturation, viability and storage [5, 6]. In endocytosis, the main role is assigned to lysosomes, containing a variety of hydrolytic enzymes [20]. Immunoexpression of most currently examined lysosomal enzymes was previously reported in physiological and pathological stages in both human [1, 20] and animal studies [3, 5, 11]. Their activity and expression is regulated by androgens and estrogens, especially in efferent ducts, where epithelial cells are responsible for the fluid absorption [5, 6, 20]. On the other hand, Mayoraga and Bertini [17] found that the epidydymal cathepsin D activity was increased after orchidectomy and decreased after testosterone treatment. Even though such a procedure was not performed in the present study, an increase of the enzyme activity may by a consequence of the testosterone level abnormality that is typical of diabetes [7, 12, 18,]. The observed changes in the enzymes' activity may be also secondary to affected endocrine regulation mediated by other circulating hormones (including insulin) and testicular lumicrine factors that enter the epididymal lumen directly from the efferent ducts [5, 6, 13]. A similar regulation was also found for other lysosomal enzymes like glucuronidase, RNase II, transforming growth factor beta, gamma glutamyl-transpeptidase III or sulfated glycoprotein 1 and 2 [9, 11].

Moreover, lysosomal enzymes disturbances were also noted in other organs of the examined group of rabbits, including arterial wall [23], brain cortex [22], gingivae [24], pancreas [15, 16], liver [25], submandibular and parotid glands [14], skeletal muscles [27], thyroid gland [26], and testis [28]. Based on epidemiological and clinical data [2, 7, 8, 10, 12, 18], the previously reported testicular enzymatic changes [28] were indicated as one of the factors that may induce male infertility. Such a hypothesis is supported by current results.

CONCLUSIONS

The obtained data suggest that the free and bound fraction activity of acid phosphatase, beta-galactosidase, beta-*N*-acetyl-glucosaminidase, as well as cathepsin B, D and L was alerted in rabbit epididymis in the course of experimentally induced diabetes mellitus.

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SUMMARY

Diabetes is a metabolic disease caused by inappropriate secretion and/or function of insulin, which may disturb morphology and function of various internal organs. The aim of the study was to evaluate the activity of selected lysosomal enzymes. The study was undertaken on male New Zeland white rabbits. Diabetes was induced by intravenous injection of alloxan, and animals were examined on days 21, 42, 90 and 180. The activity of the free and bound fraction of acid phosphatase, beta-galactosidase, beta-*N*-acetyl-glucosaminidase, cathepsin B, D and L, as well as lipase and sulphatase was spectrophotometrically measured in homogenates of the epididymal body. Disturbances of acid phosphatase, B-galactosidase, beta-*N*-acetyl-glucosaminidase, cathepsin B, D and L were reveled. Lack of significant changes was found for lipase and sulphatase. It could be concluded that diabetes mellitus alerts the activity of selected epididymal lysosomal enzymes.

STRESZCZENIE

Cukrzyca jest chorobą metaboliczną spowodowaną nieprawidłowym wydzielaniem insuliny, w przebiegu której mogą wystąpić zaburzenia funkcji i morfologii wielu narządów. Celem pracy była ocena aktywności wybranych enzymów lizosomalnych w najądrzu królika w cukrzycy doświadczalnej (alloksanowej) w 21, 42, 90 i 180 dniu choroby. W homogenatach najądrza spektrofotometrycznie oznaczono aktywność frakcji wolnej i związanej fosfatazy kwaśnej, beta-galaktozydazy, beta-*N*-acetylo-glukozaminidazy, katepsyn B, D i L, lipazy oraz sulfatazy. W badanej populacji zwierząt stwierdzono istotne zmiany aktywności fosfatazy kwaśnej, beta-galaktozydazy, beta-*N*-acetylo-glukozaminidazy oraz katepsyn B, D i L. Natomiast aktywność lipazy i sulfatazy nie wykazywała znamiennych różnic. Otrzymane wyniki wskazują na wpływ cukrzycy na aktywność enzymów lizosomalnych w najądrzu.