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tytuł pracy: „*Aktywność egzoglikozydaz lizosomalnych gruczołów ślinowych w przebiegu eksperymentalnie indukowanej cukrzycy typu 1*”

Summary

Insulin-related diabetes is a systemic disease. Metabolic disorders secondary to type 1 diabetes lead to the dysfunction of a number of organs, namely the salivary glands. A proper function of the salivary glands is due to the balance between the degradation and renewal of cellular and extracellular elements. Lysosomal exoglycosidases are one of groups of enzymes involved in the process of reconstruction of the salivary glands' extracellular matrix for they take part in the catabolism of its glycoconjugates. However, specific activity of HEX, its isoenzymes HEX A, HEX B as well as of GAL, MAN, FUC and GLU in the tissue of parotid and submandibular salivary glands has not been the object of analysis in scientific papers on the streptozotocin-induced diabetes.

Therefore, this paper has attempted to provide answers to the following questions:

1. Does streptozotocin-induced diabetes affect the specific activity of lysosomal exoglycosidases of the salivary glands?
2. Does the specific activity of lysosomal exoglycosidases in the reference salivary glands depend on the age of rats?
3. Does the duration of streptozotocin-induced diabetes affect the specific activity of lysosomal exoglycosidases of the salivary glands of rats?
4. Does the specific activity of lysosomal exoglycosidases depend on the type of the studied salivary gland, both in health and in the course of streptozotocin-induced diabetes?
5. Does streptozotocin-induced diabetes affect salivary gland secretory function?
6. Is there a relation between salivary gland secretory function and the specific activity of the studied lysosomal exoglycosidases?

Studies were conducted on sexually mature male Wistar rats. Animal subjects were divided into two groups: the control group (16 rats) and the study group (16 rats). Next, both main groups were divided into two subgroups (8 rats in each) depending on the count of weeks between the moment when diabetes was induced in the study group and the moment when rats were killed (week 2 and week 4). Diabetes was induced with a single intraperitoneal injection of streptozotocin (in citrate buffer pH 4.5; 50mg/kg body weight). Animal subjects in the control group were administered citrate buffer pH 4.5 (about 0.5mL) in an intraperitoneal injection. 48 hours following the administration of streptozotocin, all rats from the study group were reported to develop diabetes (tail vein blood glucose levels >250 mg/mL). Rats from both groups were killed 2 and 4 weeks following the induction of diabetes in the study group. Every time, submandibular and parotid salivary glands as well as blood from the abdominal aorta were sampled. In the serum of blood of the control group rats and the animal subjects with induced diabetes the levels of glucose and insulin were measured.

Salivary glands samples were subjected to homogenisation, sonication and centrifugation. Supernatant was collected for further studies.

In a triple assay the following were measured in supernatant: total protein concentration by the BCA method as well as the specific activity of: N-acetyl-beta-D-hexosaminidase (HEX), its isoenzymes HEX A and HEX B, β -galactosidase (GAL), α -mannosidase (MAN), α -fucosidase (FUC), β -glucuronidase (GLU) and salivary amylase (AS).

Statistica 10.0 (StatSoft) software package was used to carry out a statistical analysis. Kruskal-Wallis ANOVA by ranks test was employed to compare dependent variables. The non-parametric Mann-Whitney U test was used to compare non-normally distributed quantitative variables as an assessment of both groups. Also, the non-parametric Spearman's rank correlation coefficient was calculated. The adopted value of statistical significance was $p < 0.05$.

In the 2nd week of the experiment in the homogenates of parotid salivary glands of rats with streptozotocin-induced diabetes:

- the medians of specific activity of GAL, MAN, FUC and GLU were significantly higher compared to the median of specific activity of the aforementioned enzymes in parotid salivary glands of the reference group ($p=0.0046$, $p=0.0046$, $p=0.002$, $p=0.0046$, respectively)
- the median of specific activity of GLU was significantly higher compared to the median of specific activity of the aforementioned enzyme in the homogenates of submandibular salivary glands of rats with streptozotocin-induced diabetes ($p=0.029$).

In the 4th week of the experiment in the homogenates of parotid salivary glands of rats with streptozotocin-induced diabetes:

- the medians of specific activity of HEX, HEX A, HEX B, GAL, MAN, FUC and GLU were significantly higher compared to the median of specific activity of the aforementioned enzymes in parotid salivary glands of rats with streptozotocin-induced diabetes in the 2nd week of the experiment ($p=0.029$, $p=0.0047$, $p=0.029$, $p=0.0047$, $p=0.0047$, $p=0.028$, $p=0.0047$, respectively)
- the medians of specific activity of HEX A, GAL, MAN, FUC and GLU were significantly higher compared to the median of specific activity of the aforementioned enzymes in parotid salivary glands of the reference group ($p=0.0046$, $p=0.0046$, $p=0.0046$, $p=0.001$, $p=0.0046$, respectively)
- the medians of specific activity of HEX, HEX A, GAL were significantly higher compared to the median of specific activity of the aforementioned enzymes in the homogenates of submandibular salivary glands of rats with streptozotocin-induced diabetes ($p=0.029$, $p=0.0048$, $p=0.0047$, respectively).

In the 2nd week of the experiment in the homogenates of submandibular salivary glands of rats with streptozotocin-induced diabetes:

- the medians of specific activity of HEX and HEX A were significantly higher compared to the median of specific activity of the aforementioned enzymes in submandibular salivary glands of the reference group ($p=0.0048$, $p=0.0048$, respectively)
- the medians of specific activity of HEX and HEX A were significantly higher compared to the median of specific activity of the aforementioned enzymes in the homogenates of submandibular salivary glands of rats with streptozotocin-induced diabetes ($p=0.0046$, $p=0.029$, respectively).

In the 4th week of the experiment in the homogenates of submandibular salivary glands of rats with streptozotocin-induced diabetes:

- the medians of specific activity of HEX B, MAN and GLU were significantly higher compared to the median of specific activity of the aforementioned enzymes in the homogenates of submandibular salivary glands of rats with streptozotocin-induced diabetes in the 2nd week of the experiment ($p=0.029$, $p=0.0046$, $p=0.0009$, respectively)
- the medians of specific activity of HEX and HEX A were significantly lower compared to the median of specific activity of the aforementioned enzymes in the homogenates of submandibular salivary glands of rats with diabetes induced by streptozotocin in the 2nd week of the experiment ($p=0.029$, $p=0.0047$, respectively)
- the medians of specific activity of HEX A and GAL were significantly lower compared to the median of specific activity of the aforementioned enzymes in submandibular salivary glands of the reference group ($p=0.029$, $p=0.029$, respectively)
- the median of specific activity of GLU was significantly higher compared to the median of specific activity of the aforementioned enzyme in submandibular salivary glands of the reference group ($p=0.0049$)

In the 2nd week of the experiment in the homogenates of parotid salivary glands of the reference group rats:

- the medians of specific activity of HEX and HEX A were significantly higher compared to the median of specific activity of the aforementioned enzymes in submandibular salivary glands of the reference group ($p=0.029$, $p=0.029$, respectively).

In the 4th week of the experiment in the homogenates of parotid salivary glands of the reference group rats:

- the median of specific activity of GAL was significantly higher compared to the median of specific activity of the aforementioned enzyme in the homogenates of parotid salivary glands of the reference group in the 2nd week of the experiment ($p=0.0046$)

- the median of specific activity of HEX A was significantly lower compared to the median of specific activity of the aforementioned enzyme in the homogenates of parotid salivary glands of the reference group in the 2nd week of the experiment ($p=0.028$).

In the 2nd week of the experiment in the homogenates of submandibular salivary glands of the reference group rats:

- the median of specific activity of MAN was significantly higher compared to the median of specific activity of the aforementioned enzyme in the parotid salivary glands of the reference group ($p=0.0048$).

In the 4th week of the experiment in the homogenates of submandibular salivary glands of the reference group rats:

- the median of specific activity of GAL was significantly higher compared to the median of specific activity of the aforementioned enzyme in submandibular salivary glands of the reference group in the 2nd week of the experiment ($p=0.0048$).

Based on the studies the following conclusions were drawn:

1. Streptozotocin-induced diabetes caused significant changes in the activity of the majority of the studied lysosomal exoglycosidases in the salivary glands of rats.
2. The age of a rat does not affect specific activity of the majority of the studied lysosomal exoglycosidases in the reference salivary glands.
3. Specific activity of the majority of the studied lysosomal exoglycosidases in diabetic salivary glands changes significantly as the duration of the disease progresses.
4. Specific activity of lysosomal exoglycosidases is not dependent on the type of the studied salivary gland, either in health or in the course of streptozotocin-induced diabetes.
5. Streptozotocin-induced diabetes leads to the dysfunction of parotid salivary glands, which is reflected by a significantly reduced activity of salivary amylase and reduced total protein.
6. Increased activity of HEX B may lead to the impairment of the parotid salivary gland secretory function.