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The activity of lysosomal enzymes of rabbit's gingiva mucosa during induced experimental diabetes

During diabetes there was often observed inflammation of the gingival mucosa of gingiva. In available literature, very little information has been found regarding this complication. Thus we have chosen to explore this problem.

MATERIAL AND METHODS

The investigations were carried on 56 New Zealand rabbits, adults, male, weighing about 3 kg. Diabetes was provoked by means of an i. v. injection of 10% alloxan in the dose of 100 mg/kg body mass. Seven days after giving alloxan, the level of glucose in the blood was measured. As a criteria for the presence of diabetes, a level higher than 11.1 mmol/l was set. The animals were divided into the following groups: group 1 - control group - 14 rabbits, group 2 - 21-day diabetes -15 rabbits, group 3 - 42-day diabetes -10 rabbits, group 4 - 90-day diabetes -12 rabbits, group 5 - 180-day diabetes -5 rabbits.

We then removed the gingiva's mucosa for investigations. The mucosa were frozen at -20° C. After thawing, the material was homogenized in 0.3 M saccharose in ice water at the temperature $+4^{\circ}$. The received homogen was centrifuged for 10 min in 3,200 circles/min at $+4^{\circ}$ C temperature. Hypernatant was decanted and then rotated for 20 min in 14,000 circles/min. Hypernatant was then used to fix the fraction of free lysosomal enzymes. The remaining sediment was fixed in 5 ml saccharose of 0.3 M with 0.1 % Triton X-100 and was refrigerated for 24 h at $+4^{\circ}$ C temperature. Triton X-100 was used as a factor to tear lysosomal membranes. These were then rotated for 20 min in 1,400 circles/min. Supernatant was decanted and the activity of lysosomal enzymes connecting with them, were fixed. Both supernatants showed marked protein level increase. The activity of examined lysosomal enzymes was fixed to prevent substrate disintegration and given a mcg/ml protein/1 h incubation. (1, 2, 3).

RESULTS

Table 1 shows the activity of free lysosomal enzymes of rabbit's gingiva after induced experimental diabetes. The table reveals that the activity of free katepsin B in rabbits with 21-day diabetes decreases by 65% in comparison with the control group. In other experimental groups, the activity of this enzyme is below the values of the control groups. Regarding the activity of free cathepsin D, there was recorded an increase in all experimental groups in comparison with the control group. The activity of free cathepsin L in rabbits with 21-day diabetes increases by 65% in comparison with the control group. The extreme growth of activity of this enzyme was noted in

rabbits with 42-day diabetes. In other experimental groups, the activity of free cathepsin L was much greater than in control group. Activity of free acid phosphatase in all experimental groups exceeds values noted in the control group. At the same time, the extreme value was noted in rabbits with 42-day diabetes. The activity of free \(\beta\)-galactosidase, like N-acetylo-\(\beta\)-glucosaminidase, decreased during the experiment. The activity of free lipase shows an increasing tendency, at the same time the extreme value was reached in rabbits with 42-day diabetes. It should be noted that the activity of free sulfatase decreases in each experimental group.

Table 1.	The activity of free fractions of lysosomal enzymes in the gum of rabbit in the course
	of alloxan diabetics estimated in mmol/mg/1 hour of incubation

No.	Fraction of enzymes	Group 1	Group 2	Group 3	Group 4	Group 5
1	Cathepsin B	11.46	5.12	6.06	4.85	4.50
		±5.68	±1.51	±3.07	±1.69	±1.60
2	Cathepsin D	46.01	59.12	69.45	60.61	59.02
		±10.78	±17.53	±32.40	±24.56	±9.57
3	Cathepsin L	47.51	78.36	158.35	92.26	113.06
		±13.41	±26.38	±70.38	±28.47	±38.92
4	Acid phosphatase	0.063	0.075	0.091	0.075	0.077
		±0.017	±0.019	±0.024	±0.024	±0.012
5	β-galactosidase	0.023	0.020	0.014	0.019	0.019
		±0.003	±0.009	±0.012	±0.009	±0.010
6	NAGL	0,075	0.071	0.067	0.060	0.061
		±0.027	±0.024	±0.018	±0.030	±0.019
7	Lipase	0.052	0.071	0.113	0.073	0.094
		±0.011	±0.028	±0.035	±0.022	±0.028
8	Sulphatase	0.00072	0.00054	0.00061	0.00057	0.00052
		±0.00012	±0.00010	±0.00020	±0.00025	±0.00008

Table 2 shows the activity of connecting lysosomal enzymes of rabbits gingiva mucosa during induced experimental diabetes. From this table it appears that the activity of cathepsin B of rabbits with 21-day diabetes decreased by almost 50% in comparison with the control group. In other experimental groups, there was a decreasing trend of this enzyme activity. The activity of connecting cathepsin D increases by 137% in rabbits at the beginning of this illness and later substantially surpasses values found in the control group. It should be noted that the activity of connecting cathepsin L increases two times in the first experimental group in comparison with the control group, while in other experimental groups, the activity of this enzyme keeps on a high level.

Table 2 also shows that the activity of connecting acid phosphatase increased by 58% in rabbits with 21-day diabetes in comparison with the control group, while in other experimental groups, to a lesser degree but still higher in comparison with the control group. It should be noted too that the activity of connecting \(\beta\)-galactosidase except for the experimental group 3, decreases slightly in comparison with the control group, while the activity of connecting N-acetyl-\(\beta\)-galactosidase increases in rabbits with 21-days diabetes by 93%, in comparison with the control group. In other experimental groups the activity of this enzyme was a little higher than in the control group. Our results also show that the activity of connecting lipase increases in rabbits with 21-day diabetes by 73%, in comparison with the control group and keeps on this high level throughout the experiment. In addition, the activity of connecting sulfatase in rabbits of experimental group 2 increases by 68% in comparison with the control group, while in other experimental groups, the activity of this enzyme much exceeds values from the control group.

No.	Fraction of enzymes	Group 1	Group 2	Group 3	Group 4	Group 5
1	Cathepsin B	26.30	13.64	12.22	10.34	7.54
		±21.01	±13.94	±19.96	±12.0	±6.26
2	Cathepsin D	51.50	122.05	162.86	107.63	140.38
		±17.11	±42.15	±83.64	±26.30	±29.20
3	Cathepsin L	84.61	171.01	190.29	194.71	192.65
		±20.72	±56.08	±49.08	±59.36	±41.55
4	Acid phosphatase	0.026	0.041	0.029	0.0730	0.032
		±0.026	±0.026	±0.010	±0.009	±0.021
5	β-galactosidase	0,023	0.0050	0.0055	0.0043	0.0046
		±0,003	±0.0042	±0.0034	±0.0038	±0.0017
6	NAGL	0.0054	0.027	0.015	0.015	0.016
		±0.0019	±0.029	±0.013	±0.010	±0.005
7	Lipase	0.014	0.083	0.091	0.086	0.096
		±0.010	±0.030	±0.037	±0.031	±0.025
8	Sulphatase	0.00050	0.00084	0.00095	0.00088	0.00083
		±0.00035	±0.00028	±0.00024	±0,.00030	±0.00023

Table 2. The activity of connecting fractions of lysosomal enzymes in the gum of rabbit in the course of alloxan diabetics estimated in mmol/mg proteine /1 hour of incubation

From our experiment, during experimentally induced diabetes, there was increased activity of free enzymes: cathepsin D and L, acid phosphatase, lipase, while the activity of other enzymes decreases. Regarding connecting enzymes, there was increased activity of cathepsin D and L, acid phosphatase, NAGL, lipase, and sulphatase.

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SUMMARY

The investigations were carried on 56 New Zealand rabbits, adults, male weighing about 3 kg. The animals were divided into the following groups: group 1 – control group – 14 rabbits, group 2 – 21-day diabetes – 15 rabbits, group 3 – 42-day diabetes – 10 rabbits, group 4 – 90-day diabetes – 12 rabbits, group 5 – 180-day diabetes – 5 rabbits. Diabetes was provoked by means of an i.v. injection of 10% alloxan in the dose of 100 mg/kg body mass. Seven days after giving alloxan, the level of glucose in the blood was measured. As a criteria for the presence of diabetes, a level above more than 11.1 mmol/l was set. We then removed the gingivas mucosa for investigations. The mucosa were frozen at – 20 Celcius. After thawing, the material was homogenized. From our experiment, during experimentally induced diabetes, there was increased activity of free enzymes: cathepsin D and L, acid phosphatase, lipase, while the activity of cathepsin B, B-galactosidase, NAGL, sulphatase decreases. Regarding connecting enzymes, there was increased activity of cathepsin D and L, acid phosphatase, NAGL, lipase, and sulphatase.

Aktywność enzymów lizosomalnych błony śluzowej dziąsła u królika w przebiegu cukrzycy doświadczalnej

Badanie przeprowadzono na 56 królikach rasy nowozelandzkiej białej, samcach dojrzałych płciowo o masie ciała ok. 3 kg. Zwierzęta podzielono na 5 grup: grupa 1 – kontrolna – 14 królików, grupa 2 – cukrzyca 21-dniowa – 15 królików, grupa 3 – cukrzyca 42-dniowa – 10 królików, grupa 4 – cukrzyca 90-dniowa – 12 królików, grupa 5 – cukrzyca 180-dniowa – 5 królików. Cukrzycę wywołano przez dożylną iniekcję 10% alloksanu w dawce 100 mg na kilogram masy ciała, w siedem dni po podaniu alloksanu określono poziom glukozy we krwi. Za kryterium obecności cukrzycy przyjęto wartości 11,1 mmola/l. Do badań pobierano blonę śluzową dziąseł, którą zamrażano w temp. -20C. Po rozmrożeniu materiał homogenizowano. Z przeprowadzonych badań wynika, że wzrosła aktywność w przebiegu cukrzycy doświadczalnej wolnych enzymów: katepsyny D i L, fosfatazy kwaśnej i lipazy. natomiast katepsyny B, ß-galaktozydazy, NAGL i sulfatazy zmalała. Co do enzymów związanych wzrosła aktywność katepsyny D i L, fosfatazy kwaśnej, NAGL, lipazy i sulfatazy.