LIPOLYTIC ACTIVITY OF RAT SUBMAXILLARY SALIVARY GLAND PROTEIN EXTRACTS

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1. Introduction

Glucagon-like protein fractions in acid/water extract from submaxillary salivary glands of rats, pigs, rabbits, mice and humans have been found [1,2]. Their M_r values were approx. 70 000 and 30 000. These proteins with serum 30 anti-K were giving glucagon-like responses and during intravenous injections were inducing hyperglycemia.

We described a significant in vitro release of free fatty acid and glycerol from rat adipose tissue due to the effect of rat submaxillary salivary gland protein extract in [3].

We aim to elucidate some physical and biological properties of these proteins.

2. Material and methods

The extract from rat submaxillary salivary gland was obtained as in [1].

Fractionation of submaxillary salivary gland proteins extract on Sephadex G-100 revealed 3 protein peaks. M_r values of the protein fractions from submaxillary salivary gland were determined as in [4]. We compared partition (K_d) values of tested proteins and those of tested proteins from submaxillary salivary glands. M_r values of the proteins were approx. 70 000, 52 000 and 14 000 for fractions I, II and III, respectively. The electrophoretic mobility and precipitation in saturated sodium sulphate solution indicated that the fractions resembled albumin-like proteins. Each of these proteins had an isoelectric point of ~4.0–4.5 as determined by electrofocusing in a gradient of pH 3.5–10 according to the Technical Bulletin of (LKB, Sweden).

Lipid-mobilizing activity was determined by the following method: 100 mg of epididymal fat from Wistar rats was incubated with 0.5 ml of 5% cattle albumin solution (Serva) in the Krebs-Ringer bicarbonate buffer, previously gassed for 20 min with 5% CO_2/95% O_2. To the albumin solution 2000 IU/ml of Trasylol (Bayer) was added as protease inhibitor. Lipolytic activity was calculated from the amount of glycerol and free fatty acids (FFA) released to 1 ml incubated medium by 100 mg epididymal fat under influence of protein extract during 1 or 2 h of incubation. Protein fractions and epinephrine (BDH), glucagon (Sigma), ACTH (NIH, Bethesda) at concentration 10^{-6} M were used.

3. Results and discussion

All tested proteins were investigated at pH 7, 7.5, 8 and 8.5. Maximum release of FFA and glycerol was noted during incubation at pH 8.5.

During 1–2 h of incubation all examined protein fractions demonstrated significant effect on FFA and glycerol release. The first fraction showed the most pronounced lipolytic effect especially after 2 h incubation. The effect of epinephrine, ACTH and glucagon were significantly greater than observed during incubation with investigated proteins. The effect of glucagon on lipolysis after 2 h incubation was smaller than epinephrine and ACTH effects and it resembled the influence of examined protein fractions on lipid mobilization (table 1).

It is known that submaxillary salivary glands of human and several other species contain many biologically active substances, e.g. nerve growth factor, epidermal growth factor [5,6]. Submaxillary salivary

Table 1
Effect of crude water extract and protein fractions from rat submaxillary salivary gland, epinephrine, ACTH and glucagon on release of FFA and glycerol from rat adipose tissue in vitro

	FFA (μM/g tissue)				Glycerol (μM/g tissue)			
Time of incubation	1 h		2 h		1 h		2 h	
Control (n = 6)	5.93 ± 0.13	Δ	7.06 ± 0.12	Δ	0.94 ± 0.12	Δ	1.73 ± 0.10	Δ
Crude extract		1.87		1.50		2.00		3.17
(n = 6)	7.80 ± 0.14	$P < 0.05$	8.56 ± 0.20	$P < 0.05$	2.94 ± 0.30	$P < 0.05$	4.90 ± 0.31	$P < 0.05$
Fraction		2.10		3.44		2.43		3.48
I (n = 6)	8.03 ± 0.26	$P < 0.05$	10.50 ± 0.49	$P < 0.05$	3.37 ± 0.15	$P < 0.05$	5.21 ± 0.42	$P < 0.05$
		1.97		2.40		2.24		2.36
II (n = 6)	7.90 ± 0.23	$P < 0.05$	9.46 ± 0.21	$P < 0.05$	3.18 ± 0.21	$P < 0.05$	4.09 ± 0.28	$P < 0.05$
		1.70		1.63		2.14		1.63
III (n = 6)	7.63 ± 0.19	$P < 0.05$	8.69 ± 0.24	$P < 0.05$	3.08 ± 0.20	$P < 0.05$	3.36 ± 0.38	$P < 0.05$
Control (n = 6)	6.36 ± 0.12		6.96 ± 0.22		1.04 ± 0.17		1.90 ± 0.19	
Epinephrine		5.54		15.74		4.19		6.76
(n = 6)	11.90 ± 0.85	$P < 0.05$	22.70 ± 0.75	$P < 0.05$	5.23 ± 0.34	$P < 0.05$	8.66 ± 0.34	$P < 0.05$
ACTH		5.84		13.24		4.33		7.38
(n = 6)	12.20 ± 0.17	$P < 0.05$	20.20 ± 0.44	$P < 0.05$	5.37 ± 0.71	$P < 0.05$	9.28 ± 0.26	$P < 0.05$
Glucagon		4.44		4.74		2.67		6.16
	10.80 − 0.13	$P < 0.05$	11.70 − 0.33	$P < 0.05$	3.71 − 0.12	$P < 0.05$	8.06 − 0.48	$P < 0.05$

Data are mean ± S.D. Δ, differences between experiment and control, significant P = 0.05

glands might contain glucagon-like precursor material [1,2]. Our investigation demonstrated that 3 protein fractions from submaxillary salivary glands of rats are able to release FFA and glycerol from rat adipose tissue during the in vitro incubation. Their properties and physiological role in these processes will be examined in futher experiments.

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