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INSULIN FROM RIGHT MASSIVE GLAND SALIVARY OF *RAPANA VENOSA* (VALENCIENNES 1846)

RAPANA VENOSA (VALENCIENNES 1846)'NIN SAĞ MASİF İFRAZ BEZİNDEN ELDE EDİLEN İNSÜLİN

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In this work insulin was extracted from the right massive gland salivary of Rapana venosa. The isolated insulin was analysed by p.c. and t.l.c. methods and determined by RIA technique. Its hypoglycemic activity was proved on rats. The function of gland salivary has not been yet clearly known. This research is the first report on the isolation of insulin from gland salivary. In our earlier work, insulin was isolated from hepatopancreas of R. Venosa. This work is a continuation of the investigation on the insulin of R. venosa.

Bu çalışmada insülin Rapana venosa'nın sağ masif salgı bezinden elde edilmiştir. İzole edilen insülinin kağıt ve ince tabaka kromatografisi yapılmış ve RIA tekniği ile ispatlanmıştır. Kan şekerini düşürücü etkisi sıçanlarda ispatlanmıştır. Masif salgı bezinin işlevi henüz kesin olarak aydınlatılmamıştır. Bu araştırma salgı bezinden insülinin izolasyonuna ait ilk çalışmadır. Daha önceki çalışmamızda R. venosa'nın hepatopankreasından insulin izole edilmiştir. Bu çalışma R. venosa'nın insülini üzerindeki çalışmanın devamıdır.

Keywords : *Rapana venosa; Right massive gland salivary; Insulin; Hypoglycemic activity*

***Anahtar Kelimeler**: Deniz salyangozu sağ masif ifraz bez; insülin, p.c.; t.l.c.; hipoglisemian etki*

Introduction

Insulin is a hormone secreted by pancreatic islets of Langerhans. It promotes glucose utilisation. Protein synthesis and the formation and storage of neutral lipids. Insulin is obtained naturally from various animals and recently was prepared by biosynthetic methods. It is used in the treatment of diabetes mellitus (mellitus=honey).

Insulin is a polypeptide m.w. 6000 and composed of an A-chain made up of 21 amino acids (aa) and a B-chain of 30 aa. These two molecules are joined by two sulphydryl bonds. Insulins derived from different animal sources show some difference in their amino acid sequences. But almost all the species are biologically active with approximately 25 U/mg. The normal human pancreas produces insulin of about 40 U/d.

Commercial insulin is obtained from bovine and pig. The presence of insulin was demonstrated in cat, dog, horse, rabbit and marine animals such as bonito (1), angler fish (2,3), toad fish (4), cod (5,6) and also in invertebrates as mollusca (7) and hepatopancreas of *Rapana Venosa* (8).

Insulin present in all groups of fish has been purified from pancreas islets of several species. Grant and Reid (5,6) noticed that insulin of codfish from European waters differed from American codfish not only by the amino acid composition but also by having a lower biological activity. This suggests the possi-

bility of racial variation in fish insulin and other protein hormones.

Crude insulin obtained from extracts of animal raw material was prufied on ion exchanges resin (9) or Sephadex G column (6).

Insulin was analysed by p.c. and t.l.c. (10-12). Determination of its activity was made by hypoglycemic assay on rats or rabbits.

Materials and Methods

R. venosa was obtained from Mimtur Co. of Rumeli Feneri, collected from the Black Sea at 20 m depth, stored in deep freeze at -40°C.

Extraction : 100 g right massive gland salivary of *R.venosa* was separated and extracted by the methods as follows:

1. Collip method (13): Right massive gland salivary was extracted with ethanol (96%) at 4°C by continuous stirring for 30 min. Thin mixture was filtered and the filtrate was distilled under vacuum at 30°C. The residue was extracted with 2x30 ml dichloromethane to remove oil. This mixture was centrifugated, the residue was separated and stirred for 1 h at 4°C with ethanol (80%) and then recentrifuged. The supernatant was distilled under vacuum at 30°C. The aqueous phase was separated and lyophilised.
2. Garnt-Reid method (6): 100 g right massive gland salivary was mixed with 20ml trichloroacetic acid (TCA) (10%) and the residue extracted with 300 ml of a mixture of ethanol (96%)-1 N HCl-water (37:9:4) by continuous stirring for 30 min. After centrifugation the ethanol phase was filtered and distilled under vacuum at 30°C and the residue was extracted with dichloromethane, to remove fat. TCA (5%) was added to the aqueous phase. The precipitate was washed with ether-acetone mixture(1:1). The method was modified as follows; the residue was dissolved in water and after adding $(NH_4)_2\ SO_4$ (30%) the mixture was stored overnight in a refrigerator, then centrifuged and crude insulin was separated.

Analysis:

1. PC (10,11): Paper: S&S 2043 b, Solvent system: sec. Butanol-formic acid-water (75:13:11), Reagent: Bromophenol blue 1% in ethonol (96%) saturated with mercuric chloride and rinsed with three portions of aq. 0.2% mercuric chloride solution, the first of which contained 20 ml of 0.2 M Na_2HPO_4.
2. TLC; Adsorbent: Precoated Silica Gel 60 F-254 plates (Merck), Solvent system; Butanol-acetic acid-water (3:1:1), Reagent: Ninhydrine (1%) in acetone (12) and bromophenol blue (1%) in ethanol (96%) for as p.c. (10).

Activity assays:

1. Radioimmunoassay (14) (RIA): The test was conducted according to Coat-A-Count insulin procedure (Diagnostic Products Corp.). Insulin obtained from *R. venosa* was used in a conc. of 200 µL (10 mg/ml) instead of human serum.

2. Hypoglycemic activity assay(15): In each test 5 albino rats were used weighing 200-250 g. The control group consisted of 5 rats. The animals were fasted overnight, anaesthetised with pentobartital (35 mg/kg) by an i.p. injection and then test materials were injected i.v. The animals were administrated saline or insulin bovine (0.1 UI/kg) or *Rapana venosa* insulin of right massive gland salivary in a concentration of 5 or 10 mg/kg. Blood samples were taken from tail vein before and 30, 60, 120 180, 240 and 360 min. after injections and its glucose levels were determined by employing Bayer diagnostic's glucometer 3 model 5488.

Results and Discussion

1. Rf values of the insulin obtained from right massive gland salivary of *Rapana venosa* were: 0.46 for p.c. and 0.52 for t.l.c. whereas for commercial insulin 0.44 and 0.53 respectively.
2. Hypoglycemic activity results and graphical representation are shown in Table 1 and Fig. 1. Similiar results were obtained with right salivary gland insulin of 5, 10 mg/kg. The glucose level decreased until 180-240 min and then increased as with the control insulin.
3. RIA, the specific method for determining insulin gave a result of 55

U/g. This method was especially used to determine insulin.

RIA and hypoglycemic activity assay results proved that the isolated compound from *R. venosa* was insulin.

Hypoglycemic activity of insulin obtained from hepatopancreas of *R. venosa* was published earlier (8).

In this work the same effect was demonstrated for right massive gland salivary of *Gastropoda*, the function of which has not been yet clearly known.

Table 1. Blood glucose levels after administration of insulin obtained from right massive gland salivary of *R. venosa* by the methods of Collip and Grant-Reid

Time (min).	Glucose levels	
	Collip (10 mg/kg)	Grant-Reid (5 mg/kg)
0	106	102
30	67	80
60	56	66
120	67	66
240	87	77
360	79	98

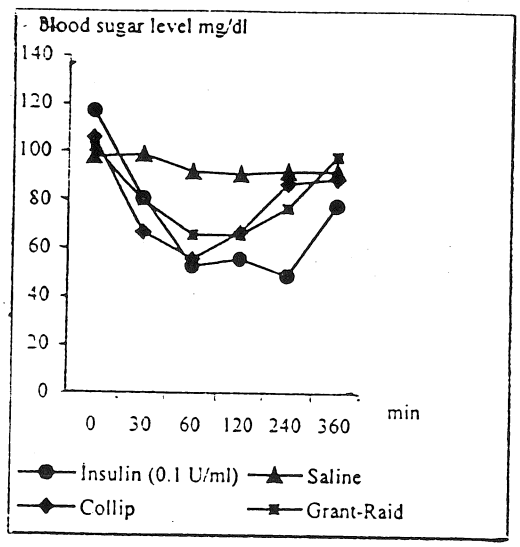

Fig.1. Graphical representation of hypoglycemic activity of right massive gland salivary of R. venosa insulin obtained by two methods

In our earlier work (16) enzyme content of right massive gland salivary was demonstrated.

This is the first record of the extraction of insulin from an unusual organ as massive gland salivary.

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