LOCALIZATION OF INSULIN-LIKE PROTEIN IN SUBMAXILLARY GLANDS OF SOME LABORATORY ANIMALS

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It has recently been shown that the submaxillary glands of mice and rats contain an insulin-like protein (ILP), which is located in the cells of the granular portions of the salivary tubules [2-4, 6, 7]. It has been suggested that the submaxillary glands participate in the maintenance of sugar homeostasis. Since the granular portions are found only in the submaxillary glands of rodents, the question arises whether ILP is present in these glands in other animals which have no granular portions. In other words, is the presence of ILP in the maxillary glands a common property of these structures or merely an exception, characteristic of rodents? The aims of the investigation described below were as follows: 1) to discover the regions of location of immunoreactive insulin (IRI) in the submaxillary glands of rabbits, guinea pigs, and cats (male) by Coons' immunofluorescence method; 2) to study the ultrastructure of those parts of the submaxillary glands of the above-mentioned animals in which IRI is detected; 3) to determine by a radioimmunochemical method the content of IRI in extracts of the submaxillary glands and pancreas of the same animals; 4) by disc electrophoresis to determine the electrophoretic mobility of ILP extracted from the submaxillary glands and to compare it with that of crystalline insulins (standards).

EXPERIMENTAL METHOD

The pancreases from six cats, 10 guinea pigs, and two rabbits (sexually mature males), deprived of food for 24 h before sacrifice, were used. To determine the localization of ILP in the pancreas, the indirect Coons' method [8, 13] was used together with appropriate controls. The glands were fixed in 4% formalin and embedded by the method in [12]. For the immunofluorescence test, guinea pig antiserum against bovine insulin and rabbit antiserum against guinea pig gamma-globulins, labeled with fluorescein isothiocyanate (FITC), were used. Guinea pigs were immunized by the scheme described previously [4]. Sections were studied under the ML-2B luminescence microscope.

Material for electron microscopy was fixed in 2.5% glutaraldehyde, postfixed with OsO_4, and embedded in Araldite by the standard method. Ultrathin sections were stained with lead citrate and examined in the IEM electron microscope (Japan) with a voltage of 100 V.

For radioimmunochemical determination [9] of IRI in extracts of the pancreas, the kit of reagents and instructions for their use from the firm CEA-Sorin (Italy) were used. Extracts of ILP were obtained by the method in [11], as used for the extraction of insulin from the pancreas. Disc electrophoresis was carried out in 15% polyacrylamide gel [1].

EXPERIMENTAL RESULTS

It was shown by the Coons' method that intensive luminescence is present in the cells of the striated portions of the salivary tubules of the submaxillary glands in all species of animals tested (Fig. 1). Luminescence was absent in the control preparations, confirming the specificity of the reaction and the presence of ILP (more exactly, of IRI) in these organs.

Under the electron microscope cells of the striated portions revealed a unique organization. In the basal parts there were numerous deep invaginations and branches of the basal plasma membrane. Between these

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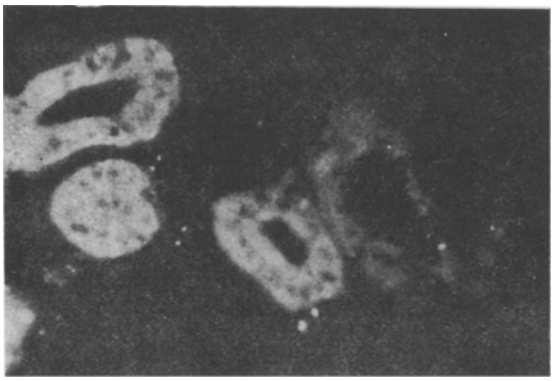

Fig. 1. Pancreas of a cat (male). Immunofluorescence method. Immune serum-FITC.

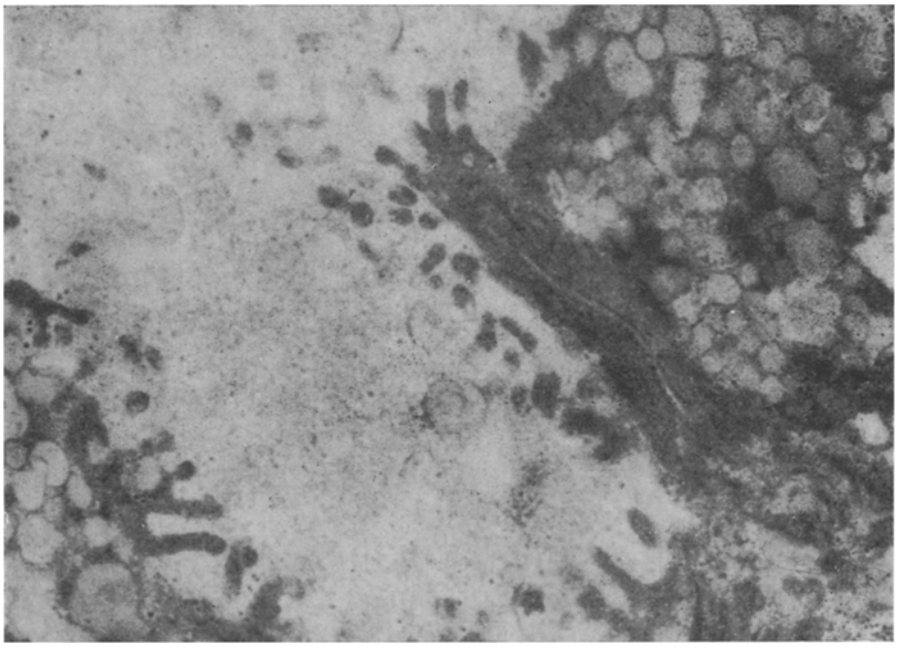

Fig. 2. Pancreas of a cat (male). Electron micrograph of apical part of cell of striated duct, magnification: 15,000× 2.2.

folds there were many mitochondria, showing different degrees of swelling. In the apical parts of the cells tiny vacuoles filled with homogeneous material of average or low electron density were observed (Fig. 2). The possibility cannot be ruled out that ILP was localized in such vacuoles. This is all the more likely because electron-histochemical studies of the submaxillary glands of mice [2, 4] have revealed ILP within the secretory granules.

The results of radioimmunochemical determination of IRI in extracts of the pancreas and in the blood serum were as follows (Table 1). First, IRI was present in the salivary glands. This fact was not previously

TABLE 1. Results of Determination of IRI Content in Extracts of Submaxillary Glands and Pancreas (M ± m)

Species of animals (males)	Number of IRI in glands, microunits	
	submaxillary gland	pancreas
Cats	715,0±0,5	26092,0±0,2
Rabbits	2859,0±0,5	52571,0±0,2
Guinea pigs	3316,0±1,2	40276,0±0,4

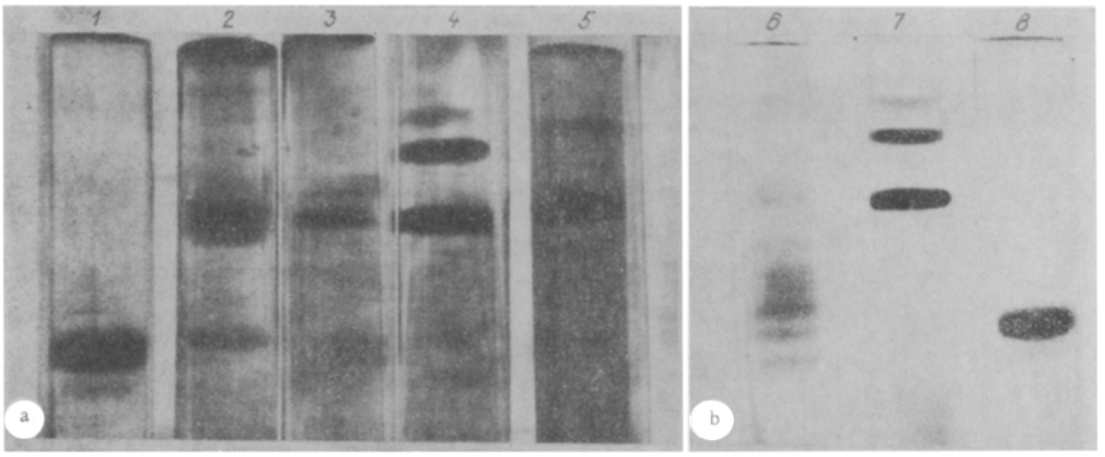

Fig. 3. Disc electrophoresis in polyacrylamide gel. 1) Standard bovine crystalline insulin; 2) ILP isolated from rabbit submaxillary gland; 3) ILP isolated from cat submaxillary gland; 4) standard human crystalline insulin; 5) insulin isolated from cat pancreas; 6) ILP isolated from guinea pig submaxillary gland; 7) standard human crystalline insulin; 8) standard bovine crystalline insulin.

known. It was also shown that the content of this substance in the submaxillary glands (per gram wet weight) is smaller than in the pancreas: 36.4 times in cats, 18.3 times in rabbits, and 12.1 times in guinea pigs. Meanwhile, much less IRI was found in the blood serum than in the pancreas: 63.8 times less in guinea pigs and 68.1 times less in rabbits. These results indicate that the submaxillary glands can selectively accumulate or synthesize IRI. The results of quantitative determination of IRI, in the writers' view, are relatively accurate, for kits of reagents designed for use for the determination of IRI in human blood serum and plasma were used. Other workers [10, 14] have observed that different insulins differ in their degree of binding with the antibodies included in the kits of reagents. Since no data are available on the fraction of binding with antibodies for the IRI which were investigated, it is possible that some correction will be required in future. However, the fact that IRI was present in the submaxillary glands of the experimental animals is of fundamental importance.

The use of the method of electrophoresis in polyacrylamide gel showed that ILP of the cat submaxillary glands has an electrophoretic mobility which corresponds to that of standard human insulin and of insulin extracted from the pancreas of the same animal. Rabbit ILP gave two bands on electrophoresis, one at the level of the lower (and brightest) band of standard human insulin, the other at the level of standard bovine insulin. Guinea pig ILP gave over 10 bands on disc electrophoresis, some at the same levels as human and bovine insulin (Fig. 3). The presence of several bands in the columns of gel during electrophoresis of standard and extracted insulins and ILP is evidence of the existence of insulins in different conformational states of their molecules, the possibility that insulin may bind with other substances (proteins or sialic acids) [5], and differences in their degree of degradation.

The results thus show that the submaxillary glands of cats, guinea pigs, and rabbits (males) contain ILP. It was shown by the immunofluorescence method that this substance is contained in cells of the striated portions of the salivary tubules. ILP is probably localized in small vacuoles distributed mainly apically. The content of IRI per gram wet weight of the gland was determined by the radioimmunochemical method, which showed that its content is lower in the submaxillary glands than in the pancreas, but considerably higher than in the blood serum. The electrophoretic properties of ILP of cats, guinea pigs, and rabbits are on the whole similar to those of human and bovine insulins, but they have characteristic differences. The electrophoretic mobility of pancreatic insulin extracted from a male cat and of the ILP of this animal was identical.

These observations suggest that the functions of the submaxillary glands are not confined to the production of digestive enzymes. These glands evidently play an endocrine role, they are participants in insular metabolism, and they take part in the maintenance of insular homeostasis.

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