Maturation and insulin-like immunoreactivity in rat submandibular salivary glands: possible implication of G regulatory proteins

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Streptozotocin-induced diabetes is accompanied by an increase in insulin-like immunoreactivity concentration in rat submandibular salivary glands. In this study we have examined whether, in normal state, maturation is accompanied by changes in insulin-like immunoreactivity concentration of rat submandibular salivary glands. Insulin-like immunoreactivity concentrations of submandibular salivary glands were significantly higher in 11 months old rats compared with 3.5 months old control animals. A pertussis toxin pretreatment provoked an increase in insulin-like immunoreactivity, suggesting that a pertussis toxin sensitive G-protein is involved in the regulation of insulin-like immunoreactivity in the rat submandibular salivary glands.

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Maturation in salivary glands is generally associated with degenerative structural and functional changes. On the other hand, the existence of numerous similarities in the embryologic origins and histologic structures of salivary glands and the pancreas is well documented (1). So, it is not surprising that submandibular salivary glands contain a population of specialized cells related to classical endocrine cells, particularly cells containing insulin-like material (2). Moreover, few studies report that submandibular salivary glands are tissues synthesizing and/or containing an insulin-like peptide (2, 3). We have previously shown that in rats streptozotocin-induced diabetes is associated with an increase in insulin-like immunoreactivity concentration in the submandibular salivary glands (4). Since diabetes leads to degeneration and accelerated cellular aging, we have investigated whether in the normal state maturation by itself could induce changes in insulin-like immunoreactivity concentration of submandibular salivary glands. Moreover, we have examined the possible implication of G proteins in the regulation of insulin-like immunoreactivity.

Materials and methods

Animals

Normal male Wistar rats were used and separated according to their age into two groups: 11 and 3.5 months old respectively.

Pertussis toxin procedure

Male Wistar rats (3.5 months old and 350–370 g) and fed ad libitum were used. Pertussis toxin (25 μg/kg). (List Biological Laboratories, Campbell CA) or equivalent volume of vehicle (phosphate buffer) was injected into the jugular vein under light sodium pentobarbitone anaesthesia (20 mg/kg) by ip injection. Forty-eight hours later the submandibular salivary glands were removed under anaesthesia with sodium pentobarbitone (60 mg/kg).

Submandibular salivary peptide extraction

After separation from the sublingual glands, each gland was immediately homogenized in acid ethanol (15 ml of concentrated HCl in 750 ml of absolute ethanol and 235 ml of distilled water). The insulin-like peptide was extracted in 5 ml of the acid ethanol mixture. The extraction was performed for 48 h at 4°C. After centrifugation (5000 × g for 35 min at 4°C), the supernatants were collected in chilled tubes containing 200 μl of a mixture of 32 mmol/l EDTA and 107 KU/l aprotinin (Zymolren, Specia, Paris) and stored at −18°C until assay. Protein content of the precipitates were determined by the Lowry method using 1 mol/l NaOH as solvent (5). Bovine serum albumin served as the standard.
Submandibular insulin-like immunoreactivity concentration

Insulin-like immunoreactivity concentration was measured by a radioimmunological method (6) using ¹²⁵I insulin from Commissariat à l’Energie Atomique, France. Novo rat insulin was used as the standard and the anti-insulin serum was supplied by Miles Laboratories (Puteaux, France). The detection limit was 0.1 µg/l. Insulin-like immunoreactive peptide is expressed in pg per mg of protein.

Assessment of metabolic state

Just before the experimental procedure, blood samples were collected by intracardiac puncture to determine non-fasting plasma insulin and blood glucose levels. Glycaemia was evaluated by the potassium ferricyanide method using a Technicon autoanalyser (7).

Statistical methods

Data are presented as means±SEM. Differences between groups were assessed by Student’s t-test (8).

Results

Parameters of rats in different experimental groups

In 11 months old rats, blood glucose levels were significantly higher than in 3.5 months old animals (10.6±0.3 vs 8.6±0.4 mmol/l, p<0.01). Plasma insulin levels were slightly decreased (2.0±0.4 vs 3.6±0.8 µg/l in 3.5 months old rats, p<0.05).

Pertussis toxin pretreatment induced significant changes in plasma insulin and blood glucose levels: an increase in insulinemia (11.1±0.9 µg/l, p<0.001) and a decrease in glycaemia (7.3±0.2 mmol/l, p<0.01). Vehicle injection had no effect.

In 11 months old rats, body weight, submandibular salivary gland weight and protein concentrations were higher than in 3.5 months old rats (p<0.01), respectively 525±12 vs 408±8 g, 398±7 vs 346±6 mg and 65±2 vs 48±1 mg.

Insulin-like immunoreactivity concentration in submandibular glands (Fig. 1)

The insulin-like immunoreactivity concentration of submandibular salivary glands revealed a significant increase with maturation. Concentrations were 66.4±10.0 and 102.7±14.3 pg/mg protein, respectively for 3.5 months and 11 months old male rats (p<0.05).

Pertussis toxin pretreatment also provoked a marked increase in insulin-like immunoreactivity concentration in 3.5 months old rats compared with 3.5 months control animals: 660.6±88.0 and 67.5±9.9 pg/mg protein respectively (p<0.001).

Discussion

Our results indicate that insulin-like immunoreactivity concentration in the submandibular salivary glands of rats increases during maturation. Moreover, our experiments suggest that pertussis toxin-sensitive G proteins are involved in the regulation of insulin-like immunoreactivity concentration in the submandibular salivary glands.

In 11 months old male rats, a decrease in insulinemia was observed, in agreement with the age-associated decline in insulin secretory function (9, 10). A concomitant rise in blood glucose levels and in insulin-like immunoreactivity concentrations in the submandibular salivary glands was observed. Our previous studies have demonstrated an increase in insulin-like immunoreactivity concentration of submandibular salivary glands during streptozotocin-induced diabetes (4). So, a decrease in insulin secretion would enhance the synthesis and/or liberation of the insulin-like immunoreactive peptide in the submandibular salivary glands of rats. It is pointed out that alterations in G proteins have been reported in streptozotocin-induced diabetes (11). On the other hand, guanine nucleotide-binding proteins (G proteins) were recently identified in exocrine salivary glands, especially in submandibular salivary glands (12). In our experiments pertussis toxin pretreated rats show a clear hyperinsulinaemia which was accompanied by a decrease in blood glucose levels. This observation is in agreement with the hyperinsulinaemia previously reported in pertussis children patients (13). Finally, the present study shows that the inactivation of G proteins by pertussis toxin is accompanied by a rise in insulin-like concentration in the rat submandibular salivary glands.
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References


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