Secretory pattern of characteristic glycoprotein species from segments of the rat submandibular gland following methoxamine stimulation

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It is recognized that the rat submandibular gland consists of five distinct parenchymal segments, acini, intercalated ducts, granular ducts, striated ducts and excretory ducts¹⁾, and the function of these segments are different from each other. These parenchymal components can be separated from salivary glands of the rat following a microdissection method devised by us²⁾.

Using these dissociated preparations and electrophoretic techniques, we found that the functional segments of the submandibular gland of intact rats contain several glycoprotein species; some of these have a characteristic response to stimulation and are secreted into submandibular saliva in a different manner depending on the kind of drug, such as, cholinergic agonists³⁾ and α - or β -adrenoceptor agonists⁴⁾.

The present study was designed to observe the secretory pattern of these characteristic glycoprotein species in acini and convoluted granular tubules following stimulation by methoxamine, because such observations have not yet been reported. For this purpose, changes in the salivary flow rate and the composition of glycoprotein species were followed during salivation induced by an α_1 -adrenergic agonist, methoxamine.

Materials and Methods

Preparation of functional segments

Three groups, each consisting of 4 male Sprague-Dawley rats, six weeks of age, were used in these studies. Preparation of paren-

chymal components from rat submandibular glands followed the method reported previously by us²⁾. Rats which are intact or at 30 and 60 min after intraperitoneal injection of 6 mg/kg of methoxamine (Mexan., Nippon Shinyaku) were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and perfused via carotid artery with a modified Hank's solution containing 0.1% collagenase, 1.0 mM CaCl_2 and 0.1% bovine serum albumin (pH 7.4). The unilateral submandibular gland separated from the sublingual glands was immediately removed, sliced, suspended in the same solution, and incubated for 120 min at 37°C under an atmosphere of 95% O_2 –5% CO_2 . The slices were rinsed with ice-cold modified Hank's solution and then microdissected with the help of fine needles under stereomicroscopic observation and each segments of acini and convoluted granular tubules were separately collected into siliconized capillary tubes (Drummond Microcaps, 50 μl).

Collection of submandibular saliva

Seven rats for one group were fasted but given water *ad libitum* for the 24 hours prior to the collection of submandibular saliva. Each rat was anesthetized with sodium pentobarbital (50 mg/kg, i.p.), and excretory ducts of the sublingual gland were ligated after separation from the adherent tissues submandibular gland. The trachea was cannulated with a polyethylene tube (MRC, 1.2 $\times$ 2 mm). Submandibular saliva was then collected from the tip of the ductal cannula

using a capillary micropipette (Drummond Microcaps, 10 and 20 μ l) for periods of 5 min until 60 min after the onset of salivation stimulated by methoxamine (6 mg/kg, i.p.) administration. The total volume of saliva per 5 min was measured and then promptly stored in small ice-cold test tubes at -20°C until use. At the end of collection, the gland were carefully removed and weighed.

SDS-polyacrylamide micro diselectrophoresis

Each segment was dissolved in the same volume of 6% (w/v) sodium dodecyl sulphate (SDS) solution containing 10% 2-mercaptoethanol and heated at 90°C for 3 min. The protein content of each sample of saliva and segments was determined by the Lowry method with bovine serum albumin as the standard⁵⁾. One μ l of each sample containing 1% (w/v) SDS, 5% 2-mercaptoethanol and 20% glycerol was applied on a 10 μ l-capillary packed 4-40% continuous gradient polyacrylamide gel as described by Rùchel *et al.*⁶⁾ Electrophoresis was carried out at 60 V for 60 min using 50 mM tris-glycine buffer (pH 8.4) in 0.1% SDS. The apparent molecular weight of glycoproteins detected on the densitogram was estimated using myosin, β -galactosidase, phosphorylase b, albumin, ovalbumin, carbonic anhydrase, trypsin inhibitor and α -lactalbumin as molecular weight markers. Gels were stained either with 0.2% Coomassie Brilliant Blue R-250 for protein or periodic-schiff-reagent for glycoprotein. The stained gel was scanned with a Joyce-Loebl 3CS microdensitometer using a filter at 595 nm for Coomassie Blue and 550 nm for PAS. The ratio of individual peaks to the total stained area were estimated by weight.

Results and Discussion

The onset time of the salivation was 14.9 ± 0.4 min after methoxamine administration. The time course of the secretory rate and cumulative volume of the submandibular saliva secreted in response to methoxamine is shown in Fig. 1. The secretory rate was maximal at 5 to 10 min after the onset of

Fig. 1 Time course of secretory rate and accumulative volume of saliva secreted from submandibular glands by methoxamine stimulus. Each point represents the mean $\pm$ S.E. of 7 rats.

salivation and decreased gradually thereafter. The total volume of saliva secreted from the submandibular gland for a 60 min-period was 249.3 ± 23.0 μ l per rat.

Although electrophoretic analysis was carried out for all saliva samples collected at 5 min-intervals during salivation after methoxamine stimulus, profile patterns of PAS- and Coomassie Blue-stained gels applied with submandibular saliva secreted during the 5-10 min period of salivation, were shown as typical densitograms in Fig. 2. Glycoproteins stained with PAS were migrated on gel as two major bands and three minor bands. One of the two major bands corresponded to band I, which is characteristically found in acini, and the other corresponded to band III characteristically found in convoluted granular tubules, and one of the minor bands was band IV also characteristically found in acini, as previously reported²⁾. In the protein profiles stained with Coomassie Blue of the representative sample of Fig. 2(A),

Fig. 2 Typical densitograms of glycoprotein bands (A) and protein bands (B) of submandibular saliva secreted during the 5-10 min period after stimulus of salivation by methoxamine. Samples containing 0.25 μ g and 0.5 μ g as protein were applied on gels for staining protein and glycoprotein, respectively.

protein bands were 7 to 8 in number as shown in Fig. 2(B). The peak of the protein band corresponding to the glycoprotein band III was significantly higher than that of the protein band corresponding to glycoprotein band I as shown in Fig. 2(B). These results, therefore, suggest that the carbohydrate moiety is higher in band I than in band III. Similarly, the carbohydrate moiety was higher in band I than in band IV. Molecular weights of the protein bands corresponding to the glycoprotein band I, III and IV were approximately 130K, 31K and 21.5K dalton, respectively, the same as previously reported²⁾.

The time course changes of relative amounts of each main band which was collected at periodic intervals of 5 min was shown in Fig. 3. Relative amounts of glycoprotein band III increased at the early stage and reached the maximum during the 10-

Fig. 3 Time course of relative amounts of glycoprotein and protein bands in saliva secreted from submandibular glands by methoxamine stimulus. ●—● glycoprotein band, ○---○ protein band. Each point represents the mean $\pm$ S.E. of 7 rats.

15 min period after the onset of salivation and decreased rapidly thereafter. This curve of band III was almost parallel with the change in the flow rate of secretion. On the other hand, the relative amount of band I decreased to the lowest level during the 5-10 min period and increased gradually thereafter. The initial values of the relative amounts of band I and III plotted on the figure may partially reflect relative amounts of band I and III from the resting saliva filling ducts of the gland.

Based on the proof⁴⁾ that the secretion into the saliva of glycoprotein species of band III is derived mainly from convoluted granular tubules when elicited by methoxamine stimulus, an electrophoretic investigation was tried on two parenchymal segments of acini and convoluted granular tubules

Fig. 4(A) Densitograms of glycoprotein bands of convolved granular tubules at 0, 30 and 60 min after methoxamine administration. Samples containing 1 μ g as protein were applied on gels.

Fig. 4(B) Densitograms of glycoprotein bands of acinus obtained at 0, 30 and 60 min after methoxamine administration. Samples containing 1 μ g as protein were applied on gels.

which were isolated at 30 and 60 min after methoxamine administration. The results obtained are shown in Fig. 4. In the convolved granular tubules, as shown in Fig. 4(A), the glycoprotein species of band III was reduced markedly 60 min after methoxamine administration. Whereas, in the acini, glycoprotein species of band I did not show any appreciable change at 30 min, but it showed a little reduction at 60 min as shown in Fig. 4(B).

As a result of electrophoretic analyses of stimulated submandibular saliva in rats⁷⁾, it has been postulated that the characteristic proteins (α -type) secreted in response to high doses of α -adrenergic agonist could be derived mainly from the convolved granular tubules, whereas those (β -type) elicited by β -adrenergic and cholinergic agonists and low doses of α -adrenergic agonist were primarily from the acinar cells. Tsukutani⁸⁾

recently described that 15 mg/kg of methoxamine stimulated the convolved granular tubules of the submandibular glands in mice and initiated both morphological changes of the cells and degranulation of secretory granules contained in the cells about 20–30 min after the drug administration, and the extent of the degranulation increased with time. The onset time of degranulation, judged by the increase of glycoprotein band III in secreted saliva in this study, was about 20 min after methoxamine administration and agreed with the above histochemical findings in mice. Although our methods and experimental animals differ from Tsukutani *et al.*⁸⁾, concurrence of the onset time would indicate that secretory response of convolved granular cells to methoxamine took place at the early stage of stimulation, and may produce concomitant degradation of secretory granules.

The results presented here indicate that the methoxamine acts on convoluted granular tubules at an early stage accompanied by a high secretory rate and elicits the production of the glycoprotein species characteristic in its tubules, in a manner dependent on the secretory rate.

The authors consider that using α_1 - and α_2 -blocking agents, may confirm whether the secretion of glycoprotein species, band III, is surely mediated by an α_1 -adrenergic response at an adrenoceptor site of the convoluted granular tubules.

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