Effects of Substance P on Glycoprotein Secretion from Acinar Cells of the Rat Submandibular Gland

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Abstract—The action of substance P on glycoprotein secretion from acinar cells of the rat submandibular gland was described in this report. Salivation elicited by i.v. injection of 0.5 to 20 μg/kg of substance P was increased dose-dependently, and its flow rate was highest at the first 1 min. Major glycoprotein species secreted into saliva by substance P-stimulus were shown to be electrophoretically identical with those found in acini, but not granular convoluted tubules. These results support the view that substance P acts on acinar cells of the submandibular gland and stimulates secretion of saliva from the cells.

Substance P, an undecapeptide first found in the crude extract of bovine and rat hypothalamus, is a potent salivagogic substance (1, 2). It is known from immunohistological studies that substance P distributes mainly in secretory elements (3, 4) and major ducts (5) of rat submandibular gland. In addition, substance P was proposed to act on acinar and duct cells of the rat submandibular gland by analysis of electrolytes in the peptide-evoked saliva (6).

We reported previously that glycoprotein species markedly differ between acini and granular convoluted tubules of the rat submandibular gland (7) and that characteristic glycoprotein species in acini are secreted into the saliva by β-adrenergic and cholinergic agonists, whereas those in granular convoluted tubules are secreted by α-adrenergic agonists (8–10). The present study was carried out to elucidate the action site of substance P on the rat submandibular gland by comparing electrophoretic profiles of glycoprotein species in isolated functional segments of the gland with those in substance P-evoked saliva.

Male Sprague-Dawley rats, 8 weeks of age, were fasted but given water ad libitum for 24 hr prior to experiments. Methods for collecting submandibular saliva and for microdissecting parenchymal components were the same as reported previously (7–10). Briefly, rats were anesthetized with urethane (1.5 g/kg). Their trachea was cannulated with a polyethylene tube, and the ducts of adherent sublingual glands were ligated. After i.v. injection of 0.5, 1, 2, 5, 10 and 20 μg/kg of substance P (Protein Research Foundation, Osaka), saliva evoked from the submandibular glands was collected successively at intervals of 1 min until 5 min and thereafter at 5 min-intervals until 20 min after injection. The saliva was stored at −20°C until use. In order to prepare acini and granular convoluted tubules, submandibular glands which are normal or at 20 min after i.v. injection of 5 and 20 μg/kg of substance P were perfused via the carotid with a modified Hanks’ solution containing 0.1% collagenase (Sigma), 1.0 mM CaCl₂ and 0.1% bovine serum albumin (pH 7.4) at 20 min after injection. The submandibular gland was isolated, sliced and incubated at 37°C under 95% O₂+5% CO₂ gas for 120 min. The slices were then microdissected under a stereo-microscope, and their acini and granular convoluted tubules were separately collected into a capillary. Similarly, acini and granular convoluted tubules of a normal rat were prepared. Saliva, acini and granular convoluted tubules thus prepared were dissolved in 6% sodium dodecyl sulphate solution containing 10%
2-mercaptoethanol and heated at 90°C for 3 min. The protein content of these samples was determined by the Lowry method (11). One μl of the samples (0.2-1.0 μg protein) was applied on a 4-40% continuous gradient polyacrylamide gel packed in a 10 μl-capillary as described by Rüchel et al. (12) and electrophoresed at 60 V for 1 hr. The gels were then stained with Schiff's reagent and Coomassie Blue R-250 and traced at 720 and 550 nm using a microdensitometer.

Effects of substance P on the flow rate and volume of saliva elicited from the submandibular gland are shown in Fig. 1. The salivary flow rate was highest at the first 1 min with any dose tested and decreased exponentially thereafter (Fig. 1A). The time of salivation was prolonged with increase of the dose of substance P. The volumes of saliva secreted in response to substance P during 20 min after injection are represented in Fig. 1B. The extent of salivation seemed to reach a plateau at a dose of 20 μg/kg. The half-maximal dose of substance P was about 6.8 μg/kg. This value is close to the dose of 5 μg/kg described for whole saliva secretion from major salivary glands of the rat (13), but is higher than the dose of 1-2 μg/kg for the rat submandibular gland (14).

As reported previously, there is an electrophoretic difference in glycoprotein species between acini and granular convoluted tubules of the rat submandibular gland (7). Accordingly, it is possible to determine the action site of various autonomic agents on the functional segments of the gland by comparing electrophoretic patterns of glycoprotein species in evoked saliva with those of acini or granular convoluted tubules (8-10). Glycoprotein in saliva secreted in response to substance P gave two main bands, I and IV, whereas band III, which has been shown to be characteristically contained in granular convoluted tubules (7), was detected as a minor band (Fig. 2A-a). In repeated trials, these glycoprotein patterns were not altered by changes in the dose of substance P or time after salivation. Glyco-

![Fig. 1. Flow rate (A) and total volume (B) of submandibular saliva secreted in response to substance P in rats. Each point represents the mean±S.E. of 7 rats.](image-url)
protein species contained in substance P-evoked saliva were closely similar to those in acini, as seen in Fig. 2A-b. When substance P-evoked saliva was co-electrophoresed with the acinar sample, the main bands I and IV of saliva were electrophoretically identical with band (I) and band (IV) of the acinar sample, respectively (Fig. 2A-c). Bands (I), (III) and (IV) contained in acini are equivalent to proteins with an apparent molecular weight of 130K, 31K and 21.5K daltons, respectively, and the carbohydrate moiety is higher in (I) than (III) and (IV), as reported previously (7).

These results suggest that substance P acts on acinar cells and stimulates salivation from the cells. To confirm this assumption, acini were isolated from a normal rat and rats at 20 min after i.v. injection with 5 and 20 μg/kg substance P. Glycoprotein species of these samples were then electrophoretically compared. It was shown that the acinus-characteristic glycoprotein species, band I, was reduced in response to substance P, although its reduction was not distinctly different between 5 and 20 μg/kg of substance P (Fig. 2B). These results obtained in this study seem most likely to allow the conclusion that in the rat submandibular gland, the action site of substance P is on acinar cells and stimulates salivation from the cells.

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