The Streptozocin-Diabetic State Depresses Saliva Secretion Stimulated by Pilocarpine and Noradrenaline in Mice

Ikuko Kimura,* Hidetoshi Miyamoto, Fu-Jun Chen, and Masayasu Kimura

Department of Chemical Pharmacology, Faculty of Pharmaceutical Sciences, Toyama Medical and Pharmaceutical University, 2630 Sugitani, Toyama 930-01, Japan. Received September 12, 1995; accepted November 12, 1995

We investigated the influence of the streptozocin (STZ)-diabetic state on the dose–response curves for salivary flow and protein content in saliva stimulated by pilocarpine and noradrenaline in mice. The diabetic state increased the relative weights of parotid and sublingual salivary glands but not the weight of submandibular glands, despite body weight loss. In the dose–response curves, (1) the maximal responses to stimulation with pilocarpine and noradrenaline on salivary flow, and with noradrenaline on protein content in saliva, were depressed by the diabetic state, and (2) the value of the 50% effective dose for salivary flow with pilocarpine, but not with noradrenaline, was decreasingly altered by diabetic mice. These results suggest that xerostomia, one of the complications of non-insulin-dependent diabetes mellitus, is caused in part by muscarinic and adrenergic receptor dysfunction in the salivary glands.

Key words autonomic dysfunction; salivary glands; salivary flow; streptozocin-diabetic mouse; pilocarpine; noradrenaline

Diabetes mellitus is a disease involving the abnormal metabolism of saccharides, proteins and fats, which is caused by deficient insulin release or a reduced sensitivity to insulin receptors. Xerostomia is one of the complications of non-insulin-dependent diabetes mellitus (NIDDM) in human patients. Thirst is caused by a secretory dysfunction of the salivary gland and by hyperglycemia, the abnormal metabolism of water and electrolytes.1

Salivary secretion is regulated by the autonomic nervous system. The major constituents of saliva are fluid, electrolytes and some proteins.2 All secretions from the salivary glands are stimulated by neurotransmitters derived from parasympathetic and sympathetic nerves.3,4 Parasympathetic stimulation evokes a copious secretion of salivary fluid that is relatively poor in protein, whereas sympathetic stimulation evokes a low volume but high levels of proteins by degranulation from acinar and granular duct cells. The diabetic state decreases the secretory responses of the parotid glands to parasympathetic and sympathetic nerve stimulation.5,6 However, the diabetes-induced alterations in the sensitivities of the cholinergic and adrenergic receptors in the salivary glands are still unclear.

The aim of this study was to examine how the streptozocin (STZ)-diabetic state affected the dose–response curves of pilocarpine and noradrenaline on salivary flow, and by the protein content in the total saliva in mice.

MATERIALS AND METHODS

Animals Male ddY mice, 4 weeks of age (18—23 g), were injected with a bolus intravenous (i.v.) dose of 150 mg/kg STZ (Sigma, St. Louis, MO, U.S.A.), and the mice were used 4—5 weeks (24—36 g) after the injection. Blood glucose levels were measured using the glucose oxidase method by a glucose analyzer (Type II, Beckman, CA, U.S.A.). The blood glucose levels were more than 200 mg/dl after fasting for 13—14 h before the experiment. Age-matched non-diabetic male ddY mice (33—41 g) were used as controls.

Collection of Total Saliva After fasting, the mice were anesthetized with an intraperitoneal (i.p.) injection of 50 mg/kg sodium pentobarbital (Nembutal, Abbott Lab., North Chicago, IL, U.S.A.) and placed on a heating pad maintained at 37°C. A polyethylene tube (i.d.: 1.5 mm, length: 20 mm) was surgically cannulated into the trachea to maintain respiration. Total saliva was collected into a microcapillary tube (Microcaps, 20 μl, Drummond, PA, U.S.A.) placed under the tongue at 1 min intervals for 10 min after basal stimulation with the first dose of agonist (pilocarpine and noradrenaline, 0.1 mg/kg, i.v. respectively) and for another 20 min after i.v. injection with the second doses. The total volume of saliva collected for 20 min was measured. After collecting the total saliva, the bilateral submandibular, parotid and sublingual glands were carefully removed and weighed. Salivary flow rates were finally calculated as the volume of saliva elicited per mg wet weight of total salivary glands per min. Protein concentrations in saliva were determined according to the method established by Lowry et al.7

Materials Pilocarpine hydrochloride (Wako Pure Chemical, Osaka) and noradrenaline (Sankyo, Tokyo) were used as salivary secretagogues.

Statistical Analysis All data are expressed as means±S.E.M. The Student’s t-test and one way of analysis of variance (ANOVA), followed by the Scheffe multiple-comparison test, were used to determine the statistical significance at a p level of 0.05 or 0.01.

RESULTS

The Diabetic State Causes Weight Gain of the Parotid and Sublingual Glands but not the Submandibular Gland The absolute weights and relative weights per body weight (mg/g) of the submandibular, parotid and sublingual glands in STZ-diabetic mice were compared with age-matched normal mice (Table 1). The weights of the salivary glands of each did not differ between after and before drug stimulation (data not shown). The absolute weights of the parotid and sublingual glands were significantly higher in diabetic mice than those in normal mice, despite the body weight loss. The absolute weight of the
Table 1. The Weights per Body Weights (mg/g) of Salivary Glands in Streptozocin (STZ)-Induced Diabetic Mice Compared with Those of Normal Mice

<table>
<thead>
<tr>
<th>Gland</th>
<th>STZ-diabetes</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Submandibular</td>
<td>4.4±0.1 (125.2±2.4**)</td>
<td>4.4±0.1 (145.9±2.6)</td>
</tr>
<tr>
<td>Parotid</td>
<td>1.6±0.0** (45.0±0.8**)</td>
<td>1.2±0.0 (38.8±0.9)</td>
</tr>
<tr>
<td>Sublingual</td>
<td>0.8±0.0** (23.9±0.4**)</td>
<td>0.6±0.0 (21.5±0.3)</td>
</tr>
<tr>
<td>Total salivary</td>
<td>6.8±0.1** (195.1±3.0*)</td>
<td>6.2±0.1 (206.1±3.3)</td>
</tr>
</tbody>
</table>

The absolute weights (mg) of salivary glands are shown in parentheses. The body weights were 28.8±0.3g (n=94) for STZ-diabetic mice and 33.4±0.2g (n=78) for normal mice at 8—9 weeks of age. Each value represents the means ± S.E.M. ** p<0.01, and * p<0.05: significantly different from the value of normal mice by Student’s t-test.

The Diabetic State Depresses the Salivary Secretory Response to Stimulation by Pilocarpine and Noradrenaline

The salivary flow rates elicited by the first stimulation were less than those by the second stimulation both with pilocarpine and noradrenaline at 0.1 mg/kg (i.v.). The time- and dose-dependency of salivary flow rates after the second i.v. injection of pilocarpine (0.1—2 mg/kg) (Fig. 1). The total salivary gland in diabetic mice was lower, whereas its relative weight was higher than that of the control. The parotid glands of diabetic mice were opaque and waxy, while those of normal mice were translucent (data not shown). The percentage of submandibular gland weight compared to the total gland weight was the greatest among salivary glands, and its absolute weight was decreased in parallel with body weight loss.

Fig. 1. Time-Dependent Salivary Flow Rate Elicited by Increasing the Second Dose of Pilocarpine (0.1—2 mg/kg) (A) and Noradrenaline (0.1—1 mg/kg) (B), and Dose-Response Curves of Pilocarpine and Noradrenaline (C) for the Salivary Flow Rate in Streptozocin (STZ)-Diabetic (closed symbols) and Normal (open symbols) Mice

The salivary flow rate (ml/salivary glands wet weight/min) by second doses was measured for 20 min (A and B) after the first stimulation with pilocarpine and noradrenaline at 0.1 mg/kg. The dose-response curves of pilocarpine (triangles) and noradrenaline (circles) for salivary flow were obtained over 20 min following the second stimulation with pilocarpine (0.1—2 mg/kg, i.v.) and noradrenaline (0.1—3 mg/kg, i.v.) (C). All the values are means ± S.E.M. of 5—8 mice. **, ** p<0.01, and † p<0.05: significantly different versus normal mice by one-way ANOVA and then Scheffe multiple-comparison test.
1A) and noradrenaline (0.1—1 mg/kg) (Fig. 1B) were compared in STZ-diabetic and normal mice. The maximal flow rates (nl/mg salivary gland wet weight) were obtained 1—2 min after pilocarpine injection, and the flow rate decreased gradually within 20 min. In the STZ-diabetic and normal mice, pilocarpine and noradrenaline increased in a dose-dependent manner the total volumes of saliva secreted for 20 min (Fig. 1C).

The volume of saliva secretion after the injection of pilocarpine and noradrenaline in diabetic mice was markedly diminished as compared with normal mice, and was estimated to be 58.0% at 1.0 mg/kg pilocarpine and 63.3% at 0.3 mg/kg noradrenaline in normal mice. The total volumes of saliva stimulated by the submaximal dose (1.0 mg/kg) of noradrenaline were only 10% of those by 1 mg/kg pilocarpine (Fig. 1C). Therefore, the salivary flow by pilocarpine stimulation in the diabetic state was depressed to a much greater extent than that by noradrenaline stimulation.

The value of ED₅₀ (95% confidence limits) for salivary flow by pilocarpine in diabetic mice (0.28 (0.22—0.34) mg/kg) was smaller than that in normal mice (0.46 (0.40—0.51) mg/kg) (p < 0.01). The values of ED₅₀ for noradrenaline were not different between the diabetic mice (0.24 (0.20—0.29) mg/kg) and normal mice (0.23 (0.19—0.27) mg/kg).

The total protein content (5.94 ± 0.59 μg/mg salivary glands wet weight/20 min) in saliva by pilocarpine (1.0 mg/kg) stimulation was significantly lower in diabetic mice than that (9.50 ± 0.77 μg/mg salivary glands wet weight/20 min) in normal mice (Fig. 2). The total protein content in saliva by noradrenaline (0.1—3.0 mg/kg) stimulation was also lower in diabetic mice than that in normal mice. The protein content in total saliva stimulated by noradrenaline was markedly higher than that stimulated by pilocarpine in normal mice. In the diabetic state, the protein content by noradrenaline stimulation was lessened more markedly than that by pilocarpine stimulation.

**DISCUSSION**

Xerostomia, a complication to diabetes mellitus, is caused by a dysfunction of the autonomic nervous system, innervating the salivary glands. The roles of the sympathetic and parasympathetic nerves in salivary secretion are tested by their electrical stimulation. In STZ-diabetic rats, the parotid salivary flow is diminished during parasympathetic nerve stimulation compared with normal rats. Parotid secretory responses by sympathetic nerve stimulation are also reduced in STZ-diabetic rats.

However, diabetes-induced alterations in the sensitivities of cholinergic and adrenergic receptors in salivary glands are still unclear. The present results demonstrated a depression of the maximal responses in the dose—response curves: of salivary flow by pilocarpine-stimulation and of protein content in saliva by noradrenaline-stimulation in STZ-mice. Regarding the ability to secrete saliva, the affinity for cholinergic receptors in salivary glands was increased by the diabetic state, suggesting that cholinergic receptors may be upregulated in salivary glands of diabetic mice.

STZ-diabetes in rat lowered the amylase/peroxidase ratios in salivary protein composition caused by sympathetic and parasympathetic stimulation. Some proteins and polypeptides secreted from the salivary gland play an important role in keeping blood glucose at a normal level, because blood glucose remains at a high level in the parotidectomized rat and salivary peptide P-C derived from human salivary glands has an anti-hyperglycemic effect in STZ-diabetic mice. Therefore, the increase in protein content in saliva may promote the amelioration of the hyperglycemic state and may result in the recovery of xerostomia. The present results demonstrated the depression of pilocarpine- and noradrenaline-stimulated saliva secretion in STZ-diabetic mice. Coupling between saliva secretion and the autonomic nervous systems (muscarinic and adrenergic) may be necessary to sustain normal blood glucose levels.

In conclusion, xerostomia, a complication of NIDDM, is caused in part by muscarinic and adrenergic receptor dysfunction in salivary glands.

**Acknowledgment** We are grateful to Ms. Junko Mizuo for her skillful technical assistance.

**REFERENCES**

