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Immunohistochemical and ultrastructural investigation of acinar cells in submandibular and sublingual glands of rats fed a liquid diet

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ABSTRACT

In atrophic parotid glands induced by liquid diet, acinar cell apoptosis is increased while proliferative activity is reduced. This study aimed to clarify how liquid diet affects submandibular and sublingual glands, including acinar cell apoptosis and proliferation. Seven-week-old male Wistar rats were fed either a liquid (experimental group) or pellet diet (control group) from 3 to 21 days, respectively. Submandibular and sublingual glands were weighed and examined histologically, ultrastructurally, and immunohistochemically using antibodies to cleaved caspase-3 (Casp-3) and 5-bromo-2′-deoxyuridine (BrdU). Weights of submandibular and sublingual gland from the experimental group were not significantly different from controls at any time point. Histological and ultrastructural characteristics of experimental acinar cells in both glands were normal. Acinar cells in control and experimental submandibular glands were positively stained with periodic acid Schiff (PAS) and weakly stained by alcian blue (AB). In control and experimental sublingual glands, mucous acinar cells were PAS-positive and strongly AB-positive. Although Casp-3- and BrdU-positive acinar cells were identified in both glands in the experimental group, their labeling indices were not significantly different from controls. In conclusion, liquid diet in rats does not induce atrophic alterations to acinar cells, including apoptosis and proliferative activity in submandibular and sublingual glands.

1. Introduction

Increasing intake of soft foods, a feature of current dietary habits, is thought to negatively influence the craniofacial region (Hanihara et al., 1981; Varrela, 1992). To investigate these influences, an experimental model of feeding a liquid diet to animals is widely used. Parotid glands from liquid-fed animals become atrophic, acinar cells are reduced in size (Hall and Schneyer, 1964; Wilborn and Schneyer, 1970; Hand and Ho, 1981; Scott and Gunn, 1991; Takahashi et al., 2012) and acinar cell numbers decrease in the atrophic parotid glands (Johnson, 1982; Scott et al., 1990; Takahashi et al., 2012). Despite an increasing accumulation of knowledge regarding parotid glands of liquid-fed animals, few reports have investigated other salivary glands such as the submandibular and sublingual glands. Of these studies, some (Kim, 1990; Kuntsal et al., 2003), but not all (Eksrom, 1973; Mansson et al., 1990; Nakamura, 1997; Kurahashi and Inomata, 1999) reported that submandibular glands of rats fed a liquid diet showed atrophy. Although Mansson et al. (1990) and Kurahashi and Inomata (1999) demonstrated that liquid diet induced the atrophy of sublingual glands, other reports showed no atrophy of sublingual glands (Eksrom, 1973; Scott and Gunn, 1991; Nakamura, 1997). Therefore, the induction of atrophy in submandibular and sublingual glands by liquid diet is controversial.

Previous studies showed that apoptosis is important for atrophy of parotid glands induced by duct-ligation (Walker and Gobe, 1987; Scott et al., 1999) and regression after withdrawal of isoprenaline administration (Chisholm et al., 1995). Recently, we also demonstrated increased apoptosis and reduced proliferative activity of acinar cells in parotid glands of rats fed a liquid diet (Takahashi et al., 2012). However, no studies have investigated apoptosis and proliferation of acinar cells in the submandibular and sublingual glands of liquid-fed rats. Under some pathological conditions such as duct-ligation and isoprenaline administration, the results in parotid glands are similar to those in submandibular glands (Takahashi et al., 2000; Chisholm and Adi, 1995), while the same phenomena do not necessarily occur in duct-ligated sublingual glands (Takahashi et al., 2002). Thus, it should not be assumed that increased acinar cell apoptosis and reduced acinar cell proliferation occur in submandibular and sublingual glands of rats fed a liquid diet, as is observed in the parotid glands.

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The purpose of this study was to clarify whether liquid diet induced atrophic alterations to acinar cells in submandibular and sublingual glands, such as apoptosis and proliferation of acinar cells. This was achieved through histological, immunohistochemical, and ultrastructural analysis of submandibular and sublingual glands from rats fed a liquid diet.

2. Materials and methods

2.1. Animal experiments

This study used 32 male Wistar rats aged 7 weeks, weighing 220–240 g (Hokudo, Japan). Control rats were given a normal pellet diet and experimental rats were given a liquid diet, prepared daily by mixing two parts of water to one part of a powdered diet prepared from the normal pellet diet. Body weights of rats were measured daily. Rats were perfused with 4% buffered paraformaldehyde (pH 7.4) under pentobarbital general anesthesia on days 3, 7, 14, or 21. Four control and four experimental animals were used at each time point. Rats in both groups were deprived of food for fasting 12 h before perfusion. Rats were intraperitoneally injected with 25 mg/kg body weight BrdU 1 h prior to perfusion.

This study was approved by the Laboratory Animal Committee of Hokkaido University (Approval No. 09-0009) and complied with the Guide for the Care and Use of Laboratory Animals of Hokkaido University.

2.2. Histology

The right submandibular and sublingual glands were excised, weighed, and placed in 4% buffered paraformaldehyde (pH 7.4) for 24 h. The fixed tissue was processed routinely and embedded in paraffin for histological and immunohistochemical examination. Paraffin sections were stained with hematoxylin and eosin (HE), periodic acid Schiff (PAS), or alcian blue (AB) (pH 2.5). In the HE sections, the area of at least 1000 acinar cells in randomly selected fields at a magnification of x200 were measured, using the image-analysis system (DS-L2, Nikon, Japan).

2.3. Immunohistochemistry

2.3.1. Caspase-3

The endogenous peroxidase in sections was blocked by 0.3% hydrogen peroxide and antigen retrieval was performed using 10 mM Tris/1 mM EDTA buffer (pH 8.8) at 100 °C. Then sections were incubated with anti-caspase-3 (Casp-3) rabbit polyclonal antibody (Asp 175, Biocare Medical, CA, USA, 1:50 dilution), anti-rabbit swine polyclonal antibody (DakoCytomation, Denmark, 1:100 dilution), and streptavidin–biotin horse-radish peroxidase complex (DakoCytomation) in turn. Peroxidase activity was visualized using 3,3′-diaminobenzidine tetrahydrochloride, and sections were lightly counterstained with hematoxylin.

2.3.2. BrdU

Sections were pretreated with 0.1% trypsin at 37 °C and 3 N HCl following endogenous peroxidase blocking. Anti-BrdU mouse monoclonal antibody (Bu-20a, DakoCytomation, 1:50 dilution) was the primary antibody and anti-mouse rabbit polyclonal antibody (DakoCytomation, 1:50 dilution) was the secondary antibody. Immunoreaction was developed and sections were counterstained as mentioned above.

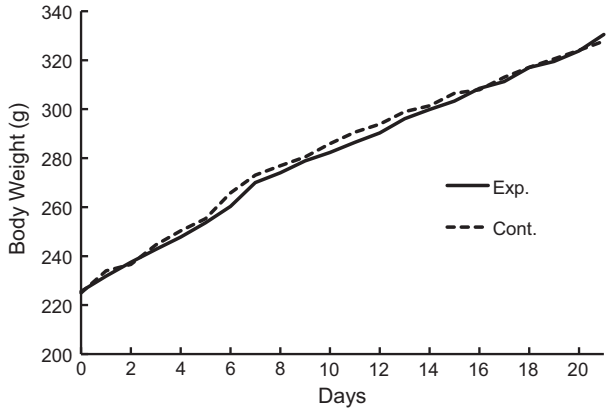

Fig. 1. The body weights of control rats fed a pellet diet (broken line, $n = 4$) and experimental rats fed a liquid diet (solid line, $n = 4$) were measured. There is no statistically significant difference in body weight (g) between the control and experimental groups at each day.

Normal rabbit or mouse serum was substituted for the primary antibody as negative controls. In the immunostained sections, at least 1000 acinar cells were counted in randomly chosen fields at a magnification of 200× using an ECLIPS 80i microscope (Nikon), and labeling indices of Casp-3 and BrdU were calculated.

2.4. Transmission electron microscopy

The left submandibular and sublingual glands were immersed in 2% buffered paraformaldehyde-1.25% glutaraldehyde (pH 7.4) for 2 h after perfusion and then rinsed with 0.05 M sodium

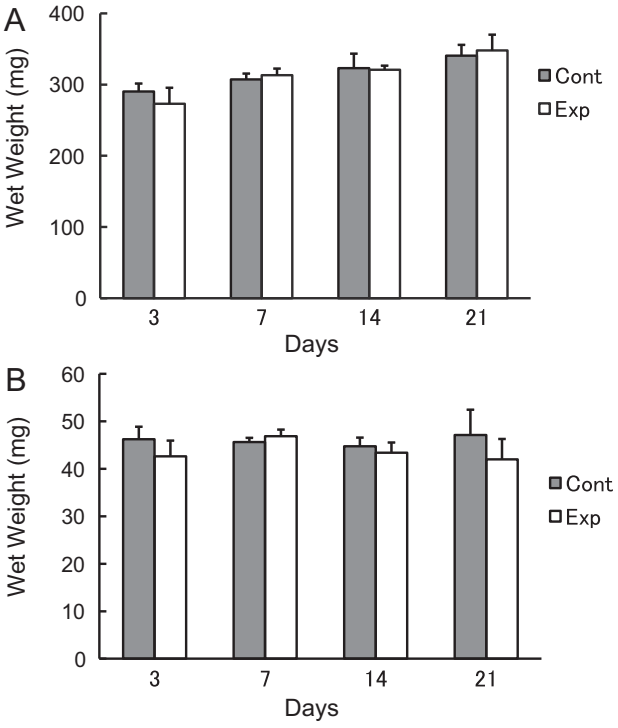

Fig. 2. The wet weights of submandibular (A) and sublingual (B) glands in control rats fed a liquid diet (filled bars, $n = 4$) and experimental rats fed a liquid diet (open bars, $n = 4$). The values are expressed as mean + SEM. No statistically significant difference was observed in wet weight (g) of submandibular and sublingual glands between the control and experimental groups.