Alteration of Masticatory Function by Diet Change Induces Stress Responses in Wistar Rats

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Abstract. The occlusion–mastication system has extradigestive functions; however, whether liquid feeding evokes stress responses remains unclear. In this study, reactions to low masticatory performance were analyzed using a diet-alteration model in Wistar rats. Seven days after the diet of the rats was changed from solid to liquid, serum epinephrine and norepinephrine concentrations were found to be elevated by 205% and 158% compared to baseline values, respectively. Superoxide production by peritoneal neutrophils was higher in rats fed with a liquid diet than in those fed with a solid diet. Serum superoxide dismutase activity (i.e. the potential to eradicate serum superoxide) was lower in rats fed with liquid than in those fed with a solid diet, indicating that the former experienced oxidative stress. Conversely, the oxidative stress was removed following reversion of the liquid diet to solid diet. These results suggest that liquid diet mastication can cause mental stress, including an oxidative stress response.

Mastication is the mechanical process of crushing and grinding foods in order to increase the surface area for efficient digestion by enzymes. It is a highly coordinated action orchestrated by the teeth, tongue, cheeks, lips, and jaws that utilizes vision, olfaction, gustation, tactaction, and temperature sense as well as memory. The trigeminal nerve plays a critical role in this system; this nerve occupies the largest area amongst all the cranial nerves according to the classic Penfield-Rasmussen diagram (1).

Mastication has other functions besides digestion, and it is believed to enhance memory function. Reduced mastication due to loss of teeth is regarded as a risk factor of dementia in elderly adults (2, 3). Spatial memory was impaired due to a decrease in acetylcholine levels in the cerebral cortex induced by tooth loss in rats (4, 5). Reduced mastication due to extraction of molar teeth disturbs spatial memory in aged senescence-accelerated mouse prone 8 mice (6). Synaptic formation of the hippocampus and parietal cortex was found to be reduced in mice with lowered masticatory performance (7, 8). The recovery of learning memory after brain infarction is delayed in rats fed with liquid meals in order to disturb the occlusion mastication function (9).

Another extradigestive function of mastication is to reduce stress responses. An increase in biting by gum-chewing may increase relaxation and prevent acute stress (10). This assumption is supported by many studies using rodent models; biting is shown to suppress acute stress responses, including inhibition of catecholamine (11-13) and corticotropin-releasing factor excretion (14), reduction of oxidative stress (15), suppression of extracellular signal-regulated kinase phosphorylation (16), and prevention of blood pressure elevation (17) and gastric ulcer formation (12).

In this study, we evaluated whether disturbance in mastication induces stress responses using a diet-alteration model in Wistar rats.

Materials and Methods

Reagents. Horse superoxide dismutase (SOD) and diethylenetriaminepenta acetic acid (DETPAPC) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Hypoxanthine was purchased from Wako Pure Chemicals (Osaka, Japan). Xanthine
oxidase was purchased from Roche Applied Science (Manheim, Germany) and 5-(2,2-dimethyl-1,3-propoxycyclophosphoryl)-5-methyl-1-pyrroline-N-oxide (CYPMPO) was purchased from Radical Research (Tokyo, Japan).

Animals and experimental design. A diet alteration model was used in Wistar rats to study the effect of disturbance in mastication on systemic stress as described previously (5, 9, 18). Male Wistar rats (nine weeks old) were purchased from Sankyo Laboratory Services Corporation (Sapporo, Japan). They were housed in groups of two per cage under controlled conditions (23°C and 12:12 h light-dark cycle at 50-60% humidity) with free access to food and water. Experiments were designed as shown in Figure 1. Briefly, the rats were fed with a solid diet for one week, and then they were divided into two groups at 10 weeks old (design day 0): a liquid diet (LD) group fed with Ensure Liquid (Abbott, Tokyo, Japan) and a solid diet (SD) group fed with a solid diet purchased from Orient Yeast Corporation (Tokyo, Japan), which had the same nutritional composition as Ensure Liquid. In a second experiment, the liquid diet was reverted to the solid diet on day 28. Each group consisted of eight rats.

The Animal Ethics and Research Committee of the Health Sciences University of Hokkaido approved the experimental protocol (2008.18). In the endpoint experiment, the rats were sacrificed on day 85.

Epinephrine and norepinephrine concentrations. Plasma epinephrine, norepinephrine, and dopamine concentrations were determined according to a previously described method (19). Briefly, plasma was collected, purified using alumina, and catecholamines were separated by high-performance liquid chromatography with an electrochemical detector (ECD-300, Eicom, Kyoto, Japan). The LODs of epinephrine, norepinephrine, and dopamine were 0.01 ng/ml, 0.01 ng/ml, and 0.02 ng/ml, respectively. The LOQs of epinephrine, norepinephrine, and dopamine were 0.02 ng/ml, 0.02 ng/ml, and 0.03 ng/ml, respectively.

Collection of neutrophils. The rats were injected intraperitoneally with 5% (w/v) casein dissolved in saline with an injection volume of 100 ml/kg. Exudative cells in the peritoneal cavity were collected 16 h after injection and centrifuged at 900 × g for 5 min at 4°C. After removing erythrocytes by hypotonic lysis, polymorphonuclear neutrophils were washed with phosphate-buffered saline. The viability of the neutrophils was greater than 95%, determined by a NucleoCounter (MS Technosystems, Osaka, Japan).

Measurement of superoxide production. Superoxide production was determined by the cytochrome c method, as described previously (20). Briefly, neutrophils were harvested by centrifugation, resuspended in Hank’s buffer solution at a density of 1×10^6 cells/ml, and the reaction was initiated by mixing 200 μl of cell suspension with cytochrome c and phorbol 12-myristate-13-acetate (PMA). The absorbance of cytochrome c reduction was recorded at 37°C for 10 min using a spectrophotometer at a wavelength of 550 nm in the presence and absence of SOD. Superoxide production was expressed in nmol–1cm–1×10^7 cells–1.

Measurement of serum SOD activity. Serum SOD activity was measured using electron spin resonance (ESR) spectroscopy, according to a method described by Nakayama et al. (21) with some modifications. In brief, blood samples were collected from the tail vein and serum was separated by centrifugation. The reaction mixture consisted of 10 μl serum, 10 mM DETAPAC, 10 mM hypoxanthine, 10 μl xanthine oxidase (0.2 U/ml), and 200 mM CYPMPO in a total volume of 70 ml. The superoxide radical intensity was measured at 30°C using ESR spectroscopy JES-REIX (Japan Electron Optics Laboratory, Tokyo, Japan), using the following conditions: sweep width=5 mT, sweep times=2 min, gain=6,300, modulation width=1.0×0.1 mT, time constant=0.1 s, center field=335.5 mT, power=10 mW, and frequency=9.43 GHz. A reference line was created using horse SOD and serum SOD activity was expressed as SOD U/ml serum.

Statistical analysis. Statistical analysis of the data was conducted using Student’s t-test in SPSS for Windows (spss Inc, Chicago, IL, U.S). In all statistical analyses, a p-value of less than 0.05 was considered statistically significant.

Results

Liquid diet increases serum catecholamine concentrations. Serum catecholamine concentrations were monitored as an indicator of stress response. As shown in Figure 2, serum
epinephrine and norepinephrine concentrations in the LD group were elevated to 205% and 158%, respectively, as compared to the SD group seven days after diet change. Elevations in catecholamine levels continued until day 28. Serum dopamine concentrations were below detectable levels in both groups.

The mean body weight of rats in the SD and LD groups was not significantly different (Figure 3), suggesting that the total intake of nutrition and water did not differ between the two groups. Blood examinations showed that blood counts and biochemical testing were within normal limits in both groups (data not shown).

**Liquid diet increases oxidative stress.** The superoxide production of neutrophils was tested. Neutrophils obtained from the LD group exhibited a higher level of superoxide production than those from the SD group. The difference became significant seven days after liquid meals were started and it continued until day 28 days after diet alteration (Figure 4A). Next, serum SOD activities were monitored (Figure 4B). Serum SOD activity decreased in a time-dependent manner, and the difference between the LD and the SD groups became significant 21 days after diet alteration. SOD activities were monitored until day 84 and the differences in activity were maintained at the same level.

**Liquid diet-induced oxidative stress is reversible.** Finally, we investigated whether the oxidative stress induced by diet alteration is reversible. As shown in experiment II of Figure 1, the liquid diet was changed back to the solid diet in the...
LD group and serum SOD activity was monitored. Serum SOD activity recovered to that of the SD group 14 days after the liquid diet was changed to the solid diet and was maintained at the same level thereafter (Figure 5).

Discussion

In the current study, diet alteration (solid to liquid) increased serum catecholamine and oxidative stress in Wistar rats. The difference in dietary characteristics caused alterations in mandibular movement, number of mastications, saliva secretion, and movements of the masticatory and other muscles (22-24). Low mastatory performance may be an important factor in maintaining stress homeostasis.

Another rodent model that interferes with mastication by cutting or extracting the molar teeth exists. Extracting or cutting molar teeth is frequently associated with lethal bleeding, which indicates that the treatment used to create this model is a stressful event. Thus, the diet-alteration model used in the current study may be superior for the evaluation of stress responses related to mastication. The oxidative stress observed in this study was equivalent to that of the restraint stress model (Figure 6), which is a gold standard rodent model for the study of stress responses (25).

In addition to catecholamine levels, oxidative stress was examined as a marker of stress response because it directly produces toxic biological molecules; oxidative stress impairs lipids, membranes, proteins, and DNA leading to unfavorable conditions including cancer, arteriosclerosis, nonalcoholic steatohepatitis, and complication of ageing (26-28). In the present study, oxidative stress was observed as a consequence of enhanced superoxide production (Figure 4A) and reduced serum SOD activity (Figure 4B). There is no practical way to measure the level of reactive oxygen species in the body. Thus, oxidative stress was assessed by examining the superoxide production of blood cells and serum SOD activity.

Recently, Xiong et al. have reported that norepinephrine stimulates production of superoxide by NADPH oxidase (29). In the current study, NADPH oxidase was found to be activated in the neutrophils of the LD group (data not shown). These observations suggest that elevated catecholamine levels stimulate the production of superoxide from neutrophils in Wistar rats of the LD group.

Amongst the catecholamines, epinephrine and norepinephrine were elevated in the serum of the LD group, suggesting that disturbance or impairment of masticatory movement may have caused psychological stress and stimulated the sympathetic adrenal medullary axis. We conjecture that in rats fed a liquid diet, reduced grinding movement of the molars leads to a reduction in sensory input from the periodontal membrane and jaw-closing musculature via the muscle spindles, promoting activation of the hypothalamus.

Figure 4. Induction of oxidative stress by diet alteration. The rats were fed with a solid or liquid diet as depicted in experiment I of Figure 1. A: The rats were sacrificed at each time point, peritoneal neutrophils were collected, and the production of superoxide was examined. B: Blood samples were collected from the tail vein at each time point and serum superoxide dismutase activity was measured. *Statistically significant at p<0.05 compared to the values for the rats on the solid diet (n 8).
The results of this study provide new insights into the understanding of human diseases that disturb mastication. Chewing gum may help to avoid unnecessary stress responses in patients treated with liquid nutrition. The preservation of appropriate occlusion and masticatory function is essential for maintaining and improving bodily health, and early treatment of patients with occlusal or masticatory dysfunction may contribute to maintaining and improving their quality of life.

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References


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