Relationship between taste-induced physiological reflexes and temperature of sweet taste

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Running head: Cold sweetness and physiological reflex

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Abstract

Excessive consumption of soft drinks has been argued as a key contributor to the epidemic of obesity in modern society. This is because they are characterized by high content of sugar, low satiety, and incomplete compensation for total energy. However, since obesity involves complex interactions of various factors, there must be more indices to link soft drink consumption and development of obesity. In this study, we have concentrated on the taste component of soft drinks and how they are consumed. We particularly investigated the temperature dependence of the indices associated with glucose metabolism such as cephalic phase insulin release and sympathetic nerve activity (SNA) of brown adipose tissue (BAT) to sweet solution since soft drinks are usually consumed in very cold temperature. Glucose solution incubated at room temperature (warm; 25 ºC) or refrigerated to 4 ºC (cold) was administered to the rats and significant rise in plasma insulin was observed at 3rd min in warm group while it was not increased until 15th min in cold group. The initial rise in plasma insulin was not coincided with a rise in blood glucose in warm group. Under the anesthesia, warm glucose solution significantly enhanced SNA of BAT while cold glucose solution exhibited no difference. The nerve response of chorda tympani also showed greater response to warm glucose solution than cold glucose solution. These results suggest cold sweet taste stimulus does not accompany expected taste-induced physiological reflexes, possibly retarding organisms' energy expenditure system.

Keywords: cephalic phase; insulin; taste; sweet; soft drink; thermogenesis; obesity.

1. Introduction

Obesity reflects complex interactions of genetic, metabolic, cultural, environmental, socioeconomic, and behavioral factors (15), and these factors ultimately lead to the imbalance of energy homeostasis. In addition, modern lifestyle such as lack of exercise and excessive caloric intake are blamed for the causes. Increased daily caloric intake has been brought about by high-fat foods, increased portion sizes, and diets high in sugars such as sucrose and fructose (3, 4, 28). Among these modern eating habits, excessive consumption of soft drinks is also viewed as one of the major contributors to obesity (1, 13, 27). In fact, epidemiologic studies in the United States have shown that increase in soft drink consumption over the past several decades is paralleled to increasing prevalence of over weight and obesity (9, 17).

The major relationship between development of obesity and soft drink consumption is unequivocally excessive caloric intake. However, this may not be the exclusive cause for the obesity and related disorders. For example, metabolism of fructose, a major ingredient in soft drinks, is also argued as a contributor (3). While energy contents, ingredients, and sugar metabolism have been focused in relation to criticizing soft drink consumption, contribution of taste component on energy metabolism has been discussed in much less extent. In particular, temperature of sweet drinks affecting physiological factors has been completely ignored. In psychophysical studies, it has been shown that temperature of foods and drinks alters perceived taste sensation. Notably, cooled sweetened solutions would be judged less sweet than warmer solutions (2, 6, 8). Considering that majority of sugar-sweetened drinks are consumed in

very cold temperature, it can be speculated that pre-absorptive physiological reflexes supposed to be induced by sweet taste should be altered by temperature.

The pre-absorptive reactions are generally separated to three phases, i.e. the cephalic, gastric, and intestinal phase. The cephalic phase responses refer to a set of physiological, endocrine and autonomic responses that result from the stimulation of sensory systems, especially in the oropharyngeal cavity (18). The cephalic phase is contrasted with the gastric and intestinal phases; it is more rapid in onset, of shorter duration and usually lower in magnitude than reactions triggered during the gastric and intestinal phases (29). Among a group of sensory inputs, taste is one of major elicitors of cephalic phase responses. The physiological significance of the cephalic phase responses on overall metabolic processes, however, has yet to be fully elucidated. However, several lines of evidence have implied essential roles of the cephalic phase responses in maintaining energy homeostasis. For example, it has been shown that intragastric glucose administration causes glucose intolerance (26). Moreover, lack of oropharyngeal stimulation diminishes postprandial thermogenesis (12, 14), which contributes energy homeostasis by converting excess nutrients to heat (22).

In present study, we have investigated possible pitfall of soft drink consumption from different point of view. This experiment will show the effect of administering refrigerated sweet solution (4ºC) over control solution simulated to room temperature (25ºC) on cephalic phase reflexes. In particular, we monitored cephalic phase insulin release (CPIR) and sympathetic discharge of interscapular brown adipose tissue. Both of these factors are involved in maintaining energy homeostasis as insulin

is the key hormone regulating anabolic metabolism and brown adipose tissue is the major organ dissipating excess energy into heat.

2. Methods

2.1. Subjects

Male Wistar rats, 8-10 weeks in age, were obtained from Nihon SLC, Shizuoka, Japan. All rats were housed in plastic cages at 24 ± 1 ºC with a 12:12-h light-dark cycle (light on 0700-1900 h). They were given free access to laboratory chow (LABO MR Stock, Nihon-Nosan, Kanagawa, Japan) and water. All experimental procedures were approved by the Gifu University Animal Care and Use Committee.

2.2 Measuring plasma insulin and glucose concentrations

Ten rats were used for the experiment. Two days before experiment, rats were anesthetized by pentobarbital sodium (40 mg/kg ip). A small incision was made at cheek and a silicon cannula was inserted to the oral cavity for administering test solutions. The cannula was sutured to the skin at the incision and the other side of the cannula was exteriorized from back of the neck. A cardiac cannula was also placed via the right jugular vein for blood sampling. The rats were individually kept with excess food and water. We found the rats ate food within 24 hours after the operation. A day before experiment, each rat was trained to drink test solutions administered through the cannula. On the test day, 12-h-fasted rats were connected to 1 ml syringe for blood

sampling. When the rats were at rest, the first blood samples of 0.1 ml were withdrawn at time -1 min for measuring basal plasma insulin and glucose levels. At time 0, the rats were either given 1 ml of 1 M glucose solution incubated at 25 ºC (warm; n = 5) or 4 ºC (cold; n = 5). Cold solution was cooled to 4 ºC because soft drinks are mostly refrigerated. On the other hand, warm solution was incubated at 25 ºC, simulating room temperature. In all cases, glucose solution was consumed within 20-30 sec. In preliminary experiments, blood samples were withdrawn at 1, 3, 5, 7, 9, 11 and 15 min after glucose solution administration. The results indicated transient rise in insulin at 3rd and 5th minutes without rise in plasma glucose levels. By 15 min, increase in both insulin and glucose levels were observed. To diminish the undesired effects of blood sampling, sampling times were therefore chosen at 3rd and 15th min to assess CPIR and glucose-triggered insulin release, respectively. When each sample was collected, the same amount of physiological salt solution blended with 20 U/ml heparin was administered.

Blood was centrifuged immediately after collection and the plasma samples were kept on ice. The glucose oxidase method was utilized for measuring plasma glucose levels (Glucose B-Test Wako, Wako Pure Chemical Industries, Ltd., Osaka, Japan). The remaining aliquots of plasma were used for measuring plasma insulin levels. Plasma insulin levels were measured by means of enzyme-linked immunoassay (Insulin Measuring Kit, Morinaga Institute of Biological Science, Inc., Kanagawa, Japan).

2.3. Neural recording procedure for the sympathetic nerve innervating BAT

Electrical activity of intercostal nerve innervating BAT was recorded (n = 6) as previously described (25). A 12-h-fasted rat was anesthetized by an intraperitoneal administration of α-chloralose-urethane solution (50 mg/kg and 500 mg/kg, respectively). The rat was placed in the prone position. A small incision was made above the scapula and interscapular BAT was partially separated from the muscle below. Five intercostal nerves, which contain sympathetic nerves entering BAT, were identified and one of the five nerve branches was cut. When isolating the nerve, care was taken to make sure the nerve was not associated with blood vessels or passing through BAT to the skin. The isolated nerve was placed on a pair of silver-silver chloride wire electrodes while the nerve branch was kept in mineral oil to prevent dehydration. The original signal of efferent mass discharges was amplified and filtered (low cut at 150 Hz; high cut at 10k Hz). The amplified signal was converted to digital signal by Power Lab (ADInstruments, Sydney, Australia) and it was then recorded on a computer through the recording software (Chart V5.0.1, ADInstruments, Sydney, Australia). The sampling rate was set at 20k Hz. Spikes above a threshold voltage level set just above background were counted by Spike Histogram (version 5, ADInstruments, Sydney, Australia).

When preparation was completed, the baseline activity was recorded for 30 min prior to initial stimulation. The tongue was gently extended with a hook and warm glucose solution was applied for 20 s at a constant rate of 10 ml/20 s by a gravity-flow system and the nerve activity was recorded for 20 min. After warm glucose stimulation, the tongue was rinsed with warm distilled water (25 ºC) for 20 s and nerve activity was

stabilized for another 20 min. After stabilization, cold glucose solution was applied and the nerve activity was recorded for another 20 min. To confirm the constancy of reactions, the experimental protocol was repeated twice for every rat. Rate meter of Spike Histogram with a reset time of 6 s was used to observe the time course of the nerve activity. For each experimental condition, a mean spike frequency of a 5 min period of sympathetic nerve activity to BAT after stimulus application was calculated in Hz.

2.4. Neural recording procedure for chorda tympani

Chorda tympani nerve responses were recorded (n = 6) as previously described (24). A 12-h-fasted rat was deeply anesthetized by an intraperitoneal injection of pentobarbital sodium (50 mg/kg) and trachea was cannulated. The chorda tympani nerve branch was exposed through a mandibular approach and placed on a pair of silver-silver chloride wire electrodes. The nerve was covered and bathed with mineral oil to prevent drying. The electrical activity of the whole chorda tympani nerve was fed to an AC amplifier and then displayed on an oscilloscope screen. Neural responses resulting from glucose solution (1.0 M) application on the tongue were integrated (time constant 1.0 s) and recorded on a chart recorder. The height of both phasic and tonic responses of the integrated chorda tympani nerve activity, which were measured at the first peak and at 10 s after the onset of stimuli respectively, was normalized to means of responses with 0.1 M NH_4Cl being taken as unity (1.0). The stability of the nerve responses was monitored by periodic application of 0.1 M NH_4Cl. The stimulation

protocol was the same as described in BAT nerve recording except that the recording duration for chorda tympani was 5 min.

2.5. Statistical analysis

Results were expressed as mean ± SD. Statistical significance was examined by analysis of variance, with post-hoc testing by means of Duncan's multiple range tests.

3. Results

Plasma insulin and glucose levels before and after drinking glucose solution were shown in Table 1. At 3 min after glucose ingestion, the rats administered with warm glucose solution exhibited significant rise in plasma insulin compared to the basal value ($P < 0.01$) whereas cold glucose solution did not exhibit significant change. At 15 min, both warm and cold glucose solution administered group exhibited significant rise in plasma insulin levels ($P < 0.01$) over the basal values. The changes in plasma glucose were not observed at 3 min in both groups while significant elevations were observed at 15 min ($P < 0.01$).

Representative sequential rate histograms of the sympathetic nerve discharges before and after oral administration of warm and cold glucose solution were shown in Fig. 1. The rate of spontaneous discharge recorded from the sympathetic nerves innervating interscapular BAT increased after the stimulation of the tongue with warm

glucose solution (Fig. 1A). In contrast, no obvious change in the discharge rate was produced by the application of cold glucose (Fig. 1B). Summation of data from 6 independent experiments showed that oral application of warm glucose solution significantly ($P < 0.05$) enhanced spike frequency of the sympathetic nerves (Fig. 1C) while cold glucose solution did not show significant change (Fig. 1D).

The representative recordings of the integrated responses and relative magnitudes of chorda tympani nerve to stimuli were presented in Fig. 2. The nerve response to cold glucose solution was weaker in comparison to warm glucose solution (Fig. 2A). Despite the differences in applied temperature, nerve responses to NH_4Cl were relatively constant that they were used for normalization of responses to glucose solution. As shown in Fig. 2B, both phasic and tonic steady-state (measured at 10 sec after the onset of glucose solution application) responses to cold glucose solution were significantly ($P < 0.05$) lower than those to warm solution.

4. Discussion

In this study, the effects of temperature of ingested sweet solution on cephalic phase responses in relation to energy metabolism have been examined. The most major sweetener used for soft drinks is fructose, but as an experimental stimulus, we have chosen glucose as a sweetener. This is because ingested glucose is reflected promptly as blood glucose. Therefore, in the absence of blood glucose increments, we can predict the insulin response would be of a cephalic phase reaction. As for the target responses,

CPIR and the sympathetic nerve activity of BAT were measured since the disturbance of them may induce metabolic disorders such as obesity and diabetes (5, 21). The present study demonstrates that cold glucose solution does not promote these cephalic phase responses. To our knowledge, this is the first report that the effect of temperature on cephalic phase responses has been explored.

The absence of CPIR when cold glucose was administered and ingested was not due to the efficacy of glucose as a taste stimulus, since the same concentration of glucose successfully induced CPIR when it was applied at 25 ºC. In addition, the beta cells of the rats administered with cold glucose solution properly released insulin in response to elevated plasma glucose, indicating the function of the beta cells is not disturbed by the treatments. It is thus rational to conclude that the absence of CPIR to ingestion of cold glucose solution is due to its cold temperature. It has been demonstrated that loss of CPIR by intragastric administration of glucose results in glucose intolerance (26). Similarly, blocking of CPIR by somatostatin infusion deteriorates glucose tolerance (5). Considering these facts, it is highly possible that the lack of CPIR after ingestion of cold glucose would also link to impairment of glucose metabolism.

The glucose solution, when applied at 4 ºC, failed to facilitate the sympathetic nerve activity. This indicates that excess energy may not be converted to heat efficiently if sugars were ingested with cold temperature. In other words, cold sugar solutions "deceive" the physiological energy expenditure system. It has been reported that intragastric feeding, in which cephalic phase reflexes would also be omitted exhibits

significant weight gain and body fat accumulation (19, 20, 23). This phenomenon, at least by part, is brought about by calorimetric reduction, particularly the reduced post-prandial thermogenesis triggered by oropharyngeal sensations such as taste (7) and texture (10, 11). Our findings alert that the attenuation of taste-triggered thermogenesis, in addition to the high caloric intake, could be a basis for the relationship between the cold soft drinks and obesity.

The nerve responses of chorda tympani to cold glucose solution applied on the tongue were much weaker than those applied with warm solution in spite of their identical concentration. These results are consistent with the report by Nakamura et al (16), who systematically examined the temperature dependence of the chorda tympani nerve responses to various taste stimuli. It is thus probable that attenuated cephalic phase responses to cold glucose solution were caused by the reduced sweet taste information conveyed to the brain. Sugar-sweetened beverages particularly carbonated soft drinks are blamed for their high sugar content. Since these are usually served cold, they must be heavily sweetened to compensate attenuated sweetness for satisfying flavor. Therefore, cold sweet beverages and food like ice creams might not only induce blunt sense of taste, but also attenuate cephalic phase responses, forcing us to consume excessive sugar.

In summary, temperature of sweet taste stimuli impacts CPIR and sympathetic nerve activity of BAT. While oral application of warm glucose solution elicited CPIR and enhanced BAT sympathetic nerve activity, application of cold glucose solution significantly reduced these responses. The taste nerve response was also reduced by

cold glucose solution that the sense of sweet taste conveyed to the brain was attenuated. Since our observations only show acute effects of sweet taste stimulus in different temperatures, we cannot conclude that these changes in physiological reflexes actually lead to obesity in the long run. We need further investigation on how temperature of sweet taste stimulus differentiates body weight and fat composition in a longer time duration.

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Figure legends

Fig. 1. The Effect of glucose application in two different solution temperatures to the tongue surface on efferent discharge rate of the sympathetic branch innervating interscapular brown adipose tissue. Taste stimulation was applied with warm glucose solution (A and C) or with cold glucose solution (B and D). Arrows indicate stimulus application. The values were presented as means ± SD. $n = 6$. $^{**}P < 0.05$ vs. basal values.

Fig. 2. Representative recordings of the integrated responses of chorda tympani nerve to glucose solution applications (A) and the relative magnitudes of the heights of both phasic and tonic responses (B). The values were presented as means ± SD. $n = 6$. $^{**}P < 0.05$ vs. cold glucose administrated group.