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Sweet Preference in Tumor Bearing Mouse and pCREB in Its Taste Receptor Cells

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ABSTRACT

Anorexic syndrome develops in cancer patients at a high incidence, which often involves disturbances in taste and smell as well as the loss of appetite and increased satiety. It has been reported that sweet stimuli increase cAMP level in taste receptor cells. This study was conducted to determine if preference for sweet is altered in anorectic tumor mice and if this behavioral alteration correlates with the sweet signal transduction in their taste receptor cells. Mice bearing a human oral squamous cell carcinoma were subjected to unconditioned taste preference test for a sweet solution (0.2% saccharine, 50% glucose) at three different times after tumor inoculation. Tissue sections containing the circumvallate papillae of tumor bearing mouse were processed for immunohistochemistry with specific antibodies against the activated form of cAMP response element-binding protein (pCREB). Anorexia developed as along with tumor growth, and sweet preference of tumor mice tended to decline in a time dependent manner after tumor inoculation. pCREB immuno-positive nuclei in the taste buds of circumvallate papillae increased in tumor bearing mice, compared to non-tumor controls. These results suggest that preference for sweet may decrease with advanced tumor growth in this mouse model of cancer anorexia, and this reduction may correlate with a basal increase in CREB activity, which perhaps plays a suppressive role in the sweet transduction pathway in taste receptor cells of cancer subjects.

Key words: Cancer anorexia, taste preference, food intake, pCREB

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INTRODUCTION

Anorexia and cachexia is found at high incidence in cancer patients (Bruera, 1997; Tisdale, 1997 for review), and the resulting malnutrition and body weight loss affects the quality of life as well as the recovery efficiency of patients (Larkin, 1998; Inui, 1999). The pathogenesis of cancer anorexia is multifactorial and involves most of the neuronal signaling pathways modulating energy intake (see Laviano et al., 2002, for review). Altered expression, release and function of the hypothalamic feeding peptides which implicated in energy intake have been reported in tumor bearing animals (McCarthy et al., 1993; Chance et al., 1994, 1995, 1996; Lee and Jahng, 2002). The central melanocortin receptors appear to mediate the pathogenesis of cancer anorexia and cachexia, which may accompany with increased melanocortin signaling (Marks et al., 2001; Wisse et al., 2001). Cytokines produced by tumor cells have been reported to be implicated in anorexia-cachexia, and modulate the regulatory pathway for appetite and feeding control in the brain (Oliff et al., 1987; Langstein et al., 1991; Opara et al., 1995; Plata-Salaman, 1996; 2000; Plata-Salaman et al., 1998).

The ability to eat and appetite appear to be the most important factors in the physical and psychological aspects of the quality of life in cancer patients (Padilla, 1986). It was reported that cancer patients with an abnormality of taste had an increased incidence of weight loss compared with patients with normal taste, and that the likelihood of having a taste abnormality increased with increasing extent of disease (DeWys and Walters, 1975). A decreased taste symptom found in cancer patients correlated with an elevated threshold for recognition of sweet taste and a decreased threshold for bitter (DeWys and Walters, 1975; Gallagher and Tweedle, 1983). It was also reported that anorectic cancer patients were more likely to prefer lower sweetness levels than nonanorectics, but sweet foods constituted a greater percentage of their daily caloric intake (Trant et al., 1982). In a rat model of experimental cancer, a small but significant suppression was detected in the preference test for sucrose (Smith et al., 1994).

In this study, we examined if preference for sweet was altered in mice bearing a human oral squamous cell carcinoma, which showed anorexia with altered expression of the hypothalamic feeding peptides in our previous study (Lee and Jahng, 2002). The activated form of cAMP response element-binding protein (pCREB) was also examined in their taste receptor cells to determine if this anorectic tumor changes a signaling pathway in taste perception of its host.

MATERIALS AND METHODS

Animals

BALB/c strain male mice in 8 weeks of age were purchased (KRIBB, Taejeon, Korea) and maintained in a consistent environment with a 12h/12h light-dark cycle (light between 07 : 00 and 19 : 00). Mice had free access to standard laboratory food (Purina Rodent Chow, Purina Co., Seoul, Korea) and tap water (membrane filtered purified water) *ad libitum*, and cared according to The Guide for animal experiments, 2000, edited by The Korean Academy of Medical Sciences, which is consistent with NIH Guideline for the Care and Use of Laboratory Animals, 1996 revised.

Tumor inoculation

One ml of KB cell culture (5×10^7 cells/ml medium), derived from a poorly differentiated squamous cell carcinoma in the mouth floor of a 54-year-old man, was subcutaneously inoculated to mice at the left side of lower dorsal area. The control mice received 1 ml of subcutaneous saline in the same area. The food intake, body weight, and tumor weight were recorded every morning at 9 : 00 AM. Tumor weight in grams was estimated by an empirically-derived formula: length×width× 1.33/100. When the mice were sacrificed, the tumor masses were excised, weighed and then processed for H/E staining to validate the tumor development in each animal.

Taste preference test

Body weight gain was gradually reduced in tumor mice over the experimental period, however, compensatory hyperphagia not detected, compared to non-tumor mice, as we previously reported (Lee and Jahng, 2002). Three groups of tumor mice

showing 9～10% ratio of tumor mass/real body weight (total body weight-tumor weight = real body weight) were subjected to preference test for sweet. Since the growth rate of tumor mass somewhat varied depending on each mouse, those 3 groups were on 29, 38 and 45 d after tumor inoculation, respectively (n=4 in each group). Age-matching non-tumor mice were tested parallel with each tumor group, as the control groups (n=4 in each group). Unconditioned preference test was performed using a protocol reported previously (Stafstrom-Davis et al., 2001). Mice were single caged with access to two drinking bottles, given one empty and one filled with water. The bottles were switched every 24 h to train the mice to drink from either bottle position. After 5 d of drinking training, mice received one bottle containing saccharin/glucose solution (0.2% saccharin, 50% glucose) and one bottle containing water. The bottle position was switched after 24 h, and fluid intake was recorded at the end of 48 h. All data were analyzed by unpaired t-test using StatView software (Abacus, Berkeley, CA, USA).

Immunohistochemistry

Mice were sacrificed by decapitation 48 h after the end session of preference test, tongues dissected immediately and immersed in Bouin's fixative for 24 h at 4°C. Routine paraffin embedding procedure was followed after dehydration of fixed tongue tissues with a series of graded ethanol. Circumvallate papillae sections at 6μm thickness were prepared on albumin-coated glass slides, processed for immunohistochemistry. Sections were incubated with normal goat serum (NGS) to reduce non-specific protein binding, then with rabbit polyclonal antibodies against pCREB (Upstate Biotech, NY, USA) diluted 1 : 100 in 0.1 M phosphate-buffered saline (PBS; pH7.4) containing 1.5% NGS for 24 h at room temperature. Sections were incubated for 1 h with biotinylated anti-rabbit IgG (1 : 200 dilution, Vector Laboratories, CA, USA), then bound secondary antibodies were amplified with the ABC kit (Vectastain Elite Kit, Vector Laboratories, CA, USA). Antibody complexes were visualized by 5 min reaction with 0.05% of 3,3-diaminobenzidine tetrahydrochloride (Sigma Co., MO, USA) as the chromogen.

RESULTS

Food intake and body weight

Body weight of tumor mice remained significantly lower than the age-matching non-tumor control in all groups over the experimental period (Fig. 1). In spite of reduced weight gain, daily food intake of tumor mice did not significantly differ from the non-tumor control during training period, revealing

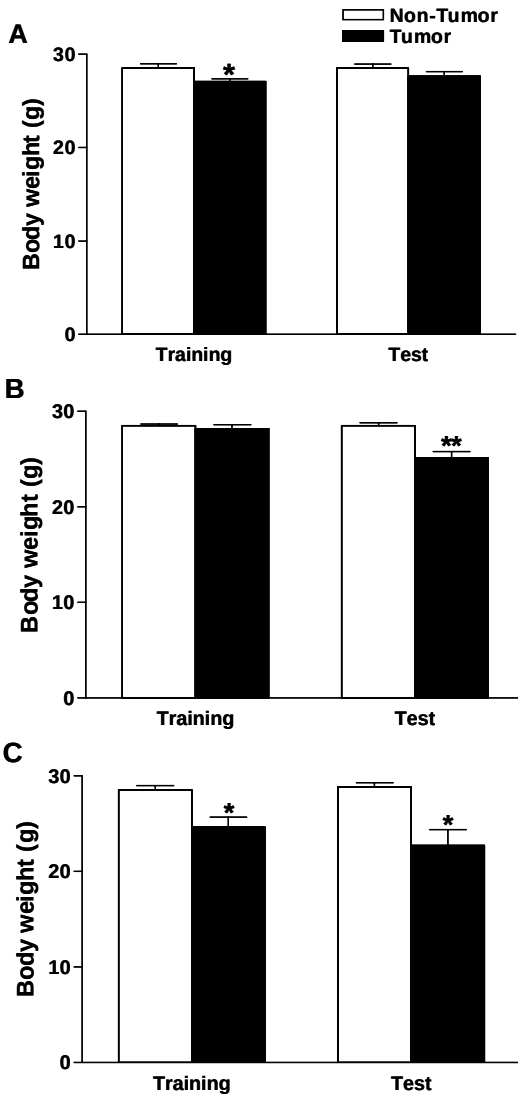

Fig. 1. Body weight of non-tumor mice and real body weight (body weight-tumor weight) of tumor mice in each group subjected to preference test at 3 different time after tumor inoculation; (A) 29 through 36 d, (B) 38 through 45, and (C) 45 through 52 d of post-inoculation. Tumor mice showed a reduction in body weight compared with the age-matching non-tumor controls during the whole experimental period. *$p < 0.05$ and **$p < 0.01$ vs. each non-tumor control.

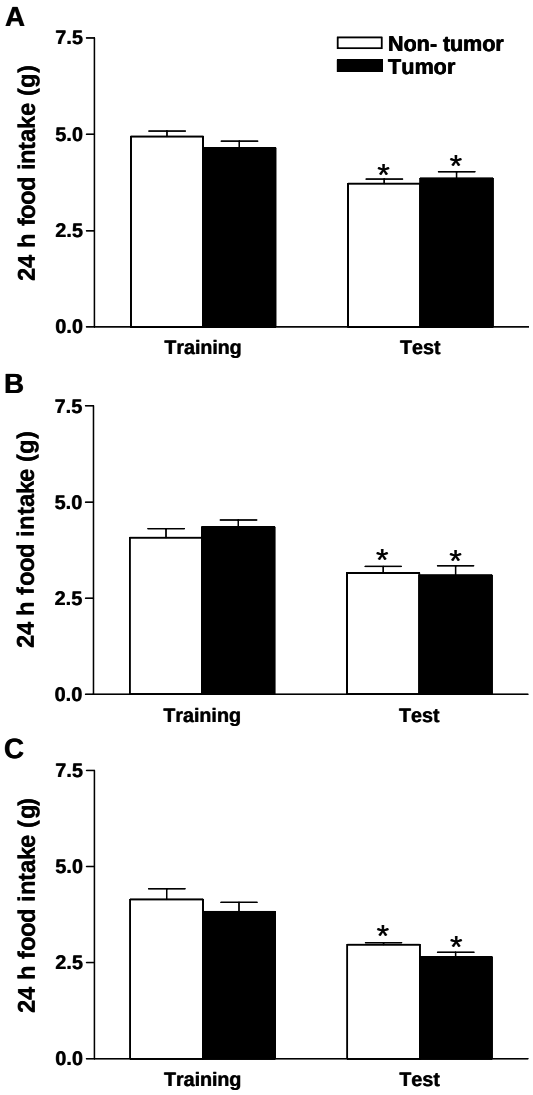

Fig. 2. 24 h food intake during each preference test; (A) 29 through 36 d, (B) 38 through 45, and (C) 45 through 52 d of post-inoculation. 24 h intake did not differ between non-tumor and tumor mice during training period, decreased in both groups during test period. $*p < 0.05$ vs. each training period.

anorexia developed in tumor mice (Fig. 2). Food intake per 100 g of body weight rather tended to be increased in the tumor group, compared to the non-tumor controls (Fig. 3). A significant reduction in 24 h food intake occurred in all groups during 48 h of preference test, compared to the training period (Fig. 2). This reduction may due to increased fluid intake as well as calories consumed from test solution, i.e. glucose per se, during the same period (Fig. 4).

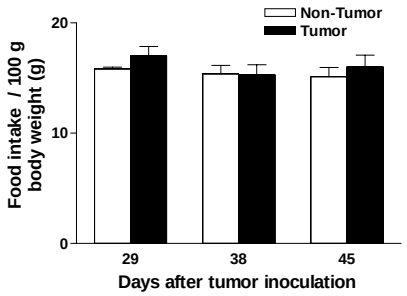

Fig. 3. 24 h food intake per 100 g of body weight (non-tumor mice) or real body weight (tumor mice) at 3 different time points after tumor inoculation, i.e. at the first day of each preference test. Food intake per 100 g of body weight tended to be larger in tumor mice, without a statistical significance.

Preference for sweet

Total fluid intake averaged for 24 h intake during test period increased in all group, compared to 24 h intake during training (Fig. 4). No significant difference was detected between non-tumor and tumor mice in preference scores for the sweet solution (saccharin/glucose) over water (Fig. 4D). However, the scores were gradually decreased in the tumor group depends on the duration of tumor bearing, not on the size of tumor mass. A gradual decrease in preference scores was also detected in the non-tumor controls, but without a statistical significance among the test groups.

pCREB immunohistochemistry

Circumvallate papillae sections were prepared 2 d after the end session of preference test. Immunoreactivities against anti-pCREB antibodies were detected in the nuclei of taste cells along the cleft of circumvallate papillae in tumor bearing mouse (Fig. 5B). On the contrary, pCREB immuno- positive nuclei were not detected in the taste cells of non-tumor control (Fig. 5A).

DISCUSSION

In this study, preference for sweet was examined in anorectic tumor mice. The preference scores for saccharin/glucose solution of tumor mice were slightly higher than of non-tumor mice through the whole

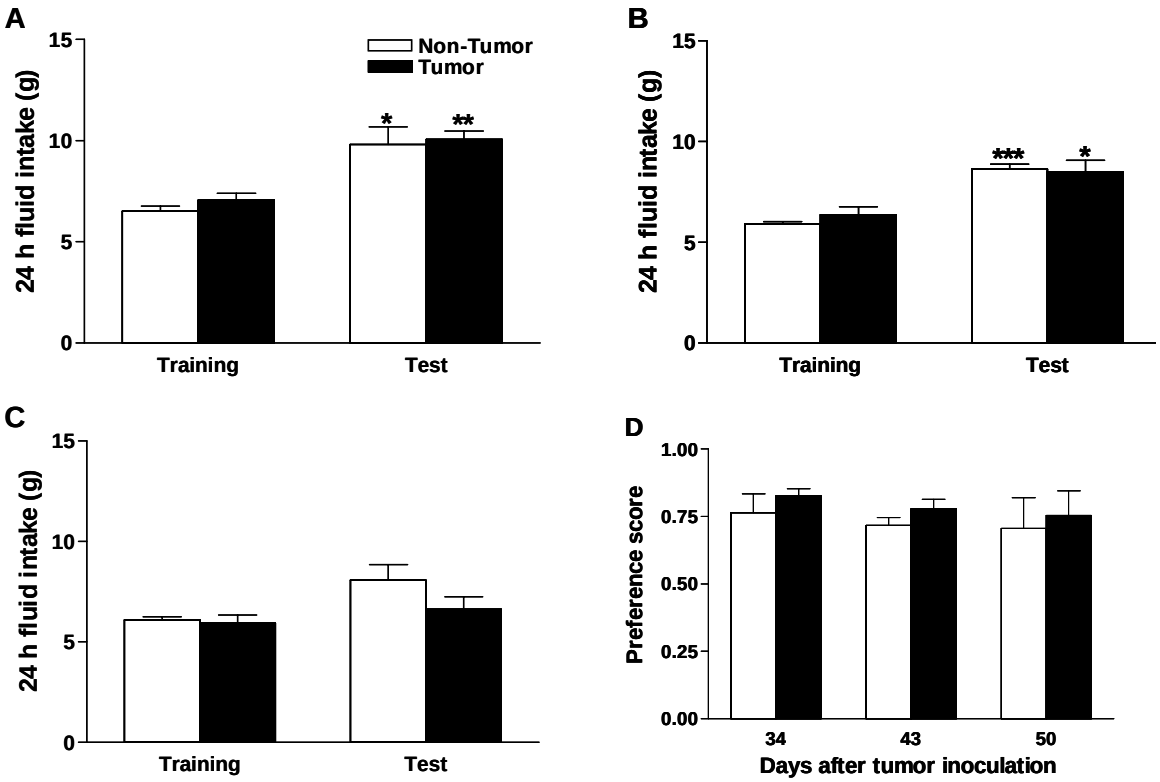

Fig. 4. 24 h fluid intake during each preference test and the preference score. Three groups of tumor mice were tested at different time points after tumor inoculation; (A) 29 through 36, (B) 38 through 45, and (C) 45 through 52 d of post-inoculation, however, they all had 9～10% ratio of tumor mass/real body weight when tested. Fluid (water) intake during training period did not differ between non-tumor and tumor mice. Total fluid intake (water+saccharin/glucose) during test period markedly increased in all groups, except the tumor group tested in the latest time point after tumor inoculation (C). A statistical significance was not found among the test groups, but the preference score tended to decline in a time dependent manner after tumor inoculation (D). $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$ vs. each training period.

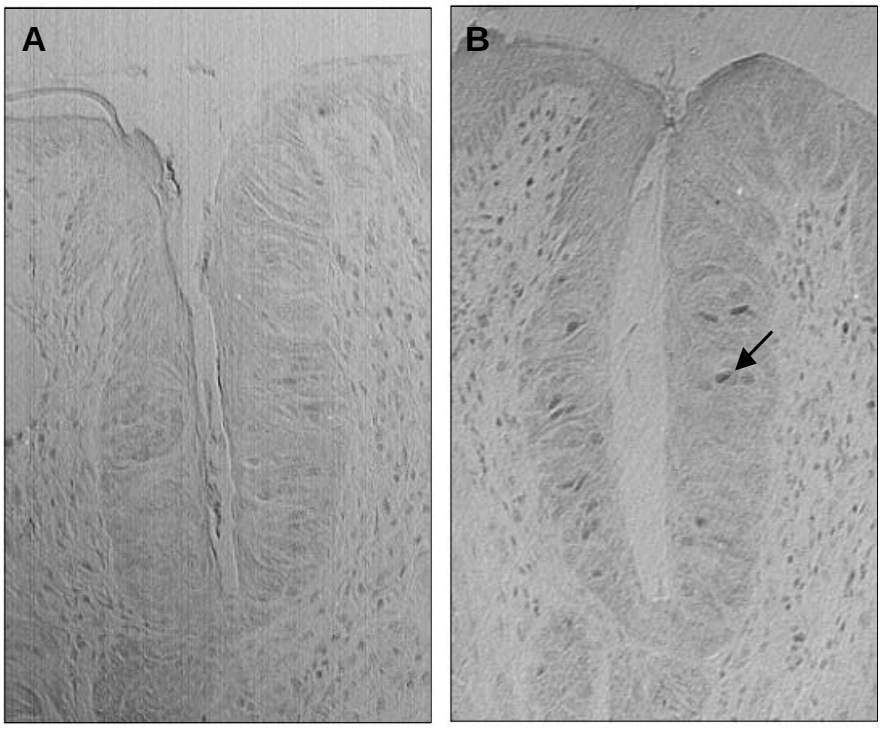

Fig. 5. pCREB immunohistochemistry of taste cells in the circumvallate papillae of non-tumor (A) and tumor (B) mouse. Tongue tissues were fixed 2 days after the end session of preference test, sectioned from paraffin block at 6μm thickness, and immunostained with polyclonal anti-pCREB antibodies. pCREB positive nuclei appeared to increase in the circumvallate papillae of tumor mouse, compared to non-tumor control.

experimental period, but a statistical significance was not found. However, a gradual decrease in preference score, which detected both in tumor and non-tumor mice in a time-dependent manner after tumor inoculation, was bigger in tumor mice. These results concur with previous report that preference for sucrose and saccharin remained still high in tumor bearing rats, but tended to decline with advanced tumor growth (Smith et al., 1994). We previously reported that the reduction in food intake started shortly after tumor inoculation, became bigger as the tumor mass grows, in the anorectic tumor mice model used in this study (Lee and Jahng, 2002). This was confirmed in the present study (data not shown), however, preference for sweet tended to decrease only at later stage of tumor develop. It was also suggested that the changes in taste preference may be secondary to the reduction in food intake (Smith et al., 1994). Taken together, it is more likely that a decrease in food intake may occur first and the following decrease in sweet preference may contribute to the reduction in food intake, consequently.

It was suggested that the decrease in food intake may be secondary to weight loss in cancer patients to match the lowered body weight (Tisdale, 2001), based on the report that food intake per kilogram of current body weight is normal in cancer patients with weight loss, although it decreased for their usual weight (Grosvenor et al., 1989). The result of intake and weight changes in the present study appears to fit this idea, however, food intake per 100 g of real body weight tended to be even larger in advanced stage of tumor growth, compared to the age-matching non-tumor controls. It is perhaps that a compensatory hyperphagia may occur with advanced tumor growth, but not in earlier time point, responding to severe weight loss, although this could not overcome the tumor-induced weight loss.

It was reported that a decreased taste symptom in cancer patients correlated with an elevated threshold for recognition of sweet taste (DeWys and Walters, 1975; Gallagher and Tweedle, 1983). cAMP is involved in signal transduction pathway for taste perception. It has recently been reported that sweet taste stimuli increase, bitter decrease, cAMP level in taste receptor cells (Cummings et al., 1996; Nakashima and Ninomiya,1999; Varkevisser and Kinnamon, 2000; see Margolskee, 2002 for review). CREB phosphorylation can be expected when cAMP level increases. Cao et al. reported for the first time the presence of CREB and its activated form, pCREB, in taste receptor cells, and that phosphorylation of CREB changed by quinine, bitter taste, stimulation (Cao et al., 2002). In this study, immunoreactivities for pCREB increased in taste cells of tumor mice in late stage of tumor develop. Taken together, it can be suggested that an increase in basal pCREB level in the taste cells of tumor mice may reveal a tonic activation of cAMP pathway with advanced tumor growth, and that this increase in pCREB can play a role in increased threshold for sweet taste of cancer subjects. In other words, due to a tonic activation of cAMP pathway in the taste receptor cells, cancer patients may have difficulties in the recognition of sweet stimuli, which reported to increase cAMP level.

In the present study, although preference for saccharin/glucose solution tended to be decreased with advanced tumor growth, however, no statistical significance was observed. It seems that saccharin, a synthetic sweetener, and sugars differently act in sweet taste transduction (Varkevisser and Kinnamon, 2000). It was also reported that mean intensity scores in taste preference tests of cancer patients directly correlated with concentration of taste stimuli (Trant et al., 1982). Saccharin/glucose solution, indeed, has been widely used for drinking tests, because animals generally show a great preference to this solution and make investigators easy to find the treatment effects. Therefore, further studies are required to define sweet preference in these tumor mice with different doses of saccharin and sugars as well.

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