Appetite Suppressing Effects of Yeast Hydrolysate on Nitric Oxide Synthase (NOS) Expression and Vasoactive Intestinal Peptide (VIP) Immunoreactivity in Hypothalamus

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INTRODUCTION

Obesity and diabetes are two common diseases that are associated not only with each other, but also with risks of cancer, hypertension, neurological disorders and cardiovascular diseases (Kopelman, 2000; King et al., 1998). Perhaps for this reason, estimates of the economic costs, prevalence, morbidity and mortality associated with modest degrees of being overweight or obese and having diabetes are on the rise (Allison et al., 1999; Mokdad et al., 2000). The development of obesity and diabetes in an individual is, respectively, characterized by the increased number and size of fat cells and by elevated blood glucose levels. They are regulated by genetic, endocrine, metabolic, neurological, pharmacological, environmental and nutritional factors (Kao et al., 2006).

Dietary modifications and increases in physical activity are the first (and the most cost effective) approaches to the treatment of obesity (Astrup, 2001); however, in many cases, a pharmacological treatment would help patients to lose weight and to reinforce their motivation in executing a dietary protocol (Fujioka, 2002). Therefore, the use of weight-lowering drugs has been proposed as a more effective treatment for weight control. However, the development of weight-lowering drugs (e.g. amphetamines) was not only disappointing in terms of efficacy, but also resulted in a number of partially severe adverse effects that ultimately led to the withdrawal of most of these compounds (Bray and Greenway, 1999).

In order to provide new discoveries and solutions, researchers chose to turn to yeast, a microorganism that mankind has long made use of. The relationship between mankind and yeast dates back many thousands of years. Yeast is a unicellular fungus and probably one of the most commercially exploited microorganisms. With more than 1000 strains of Saccharomyces cerevisiae considered GRAS (generally recognized as safe), yeast is utilized in many industries such as brewing (Casey and Inglewed, 1983), winemaking (Shinohara et al., 2000) and baking (Chell, 1997), as well as in biochemical and medical applications (Carver, 1994).

In previous studies (Kim et al., 2004), yeast hydrolysate showed anti-obesity activity in dietary obese rats, as well as anti-stress and mitigative effects in premenstrual syndrome (PMS). A number of neurotransmitters and peptide hormones reduce food intake acutely but have minimal long term effects on body weight (Morley and Flood, 1987). For example, nitric oxide synthase (NOS) inhibitors reduced food intake in rodents.
The cells were suspended in 20 mM phosphate buffer and the culture was centrifuged at 10,000 × g for 20 min. The supernatant was then passed through a 30 kDa molecular weight cut-off membrane (Satocon cassette, Sartorius, Germany) and lyophilized. A portion of the solution was freeze-dried. The sections were rinsed in phosphate buffer saline (PB) at 4 °C and frozen at −20 °C until analysed. Blood plasma levels of total cholesterol (TC), HDL cholesterol and triacylglycerol (TG) were measured using enzymatic kits (Wako Chemical Co., Osaka, Japan).

**Measurement and analysis.** Food intake and body weight were monitored every 2 days for 4 weeks. Food was withheld for 12 h before death. Under ether anesthesia, blood was collected from the aorta ventrals into tubes containing EDTA. Plasma was separated by centrifugation at 1900 × g for 15 min at 4 °C and frozen at −20 °C until analysed. Blood plasma levels of total cholesterol (TC), HDL cholesterol and triacylglycerol (TG) were measured using enzymatic kits (Wako Chemical Co., Osaka, Japan).

**Immunohistochemistry (VIP).** Immunohistochemical procedures were carried out as described previously (Kim et al., 2005). Every third section was retrieved from storage and incubated for 15 min in 3% hydrogen peroxide to eliminate endogenous peroxidase activity. The sections were rinsed in phosphate buffer saline (PBS) and incubated for 1 h in 0.05 M PBS containing 10% normal goat serum and 1% bovine serum albumin (BSA), and then in either rabbit anti-Fos (Santa Cruz, CA) or rabbit anti-Jun antibody (1:1000 dilution in PBS containing 1% BSA and 0.05% sodium azide) overnight. They were then incubated with secondary biotinylated goat anti-rabbit IgG for 1 h and with an avidin–horseradish peroxidase complex (Elite ABC kit, 1:200, Vector Laboratories, Burlingame, CA) for another 1 h. Finally, the sections were stained and dehydrated. The staining intensities of sections were assessed in a quantitative fashion according to the microdensitometric method based on optical density from an image analyser (Multiscan, Fullerton, CA).

**NADPH-diaphorase histochemistry (NOS).** NADPH-diaphorase histochemistry procedures were carried out as previously described (Scherer-Singler et al., 1983). The sections were incubated at 37 °C for 4 days in 0.1 M PB (pH 7.4) containing 0.3% Triton X-100, 0.01% nitroblue tetrazolium and 0.1% β-NADPH. Then they were washed with 0.05 M PBS and mounted on a gelatinized glass slide and dehydrated. The staining intensities of sections were assessed in a quantitative fashion according to the microdensitometric method based on optical density from an image analyser (Multiscan).

**Statistical analysis.** Data, reported as mean ± SEM, were analysed by analysis of variance (ANOVA) using SPSS for Windows (SPSS Inc., Chicago, IL). Comparisons between treatment groups were determined using the least significant difference method when ANOVA results were statistically significant (p < 0.05).

**RESULTS**

**Body weight and plasma lipids**

The body weight gain during the experimental period is shown in Table 1. The body weight gain of the BY groups (oral administration of yeast hydrolysate <10 kDa) was lower than that of the control group. In particular, the body weight gain in the BY-2 (oral administration of 1.0 g/kg) group was significantly lower than the control group. Rats orally administered yeast hydrolysate <10 kDa (BY group) exhibited a tendency toward lower weight gain, but the weight gain of the
BY-1 group was not statistically different from the control group at 21 and 28 days. The weight gain of BY-2 was also lower than that of BY-1, although there were no significant differences ($p > 0.05$) between BY-2 and BY-1. The administration of 10–30 kDa yeast hydrolysate (AY group) exhibited a tendency toward high weight gain compared with the control. Body weight gain was significantly higher ($p < 0.05$) in rats orally administered 1.0 g/kg 10–30 kDa yeast hydrolysate (AY-2) compared with the control.

Thus, the weight gain of the BY-2 group (133.0 ± 5.1 g) was significantly ($p < 0.05$) lower than the control group (150.1 ± 3.7 g) (Table 1). The food intake and food efficiency ratio were not different among the groups (data was not shown).

The effects of yeast hydrolysate on plasma lipid levels are shown in Table 2. There were no significant differences in HDL and LDL levels among the groups. However, the TG level of the BY-2 group was significantly lower than the control, BY-1 and AY-2 groups. In addition, the BY-2 group had lower levels of TC and LDL compared with the other groups, although there were no significant differences between the BY-2 and AY groups.

**Expression of NOS and immunoreactivity of VIP**

NOS in the central nervous system has been suggested to regulate food intake. In the brain and other mammalian tissues, NOS produces NO, a neurotransmitter and a biological messenger molecule. NO acts as a modulator in the control of the food intake mechanism. Therefore, the effect of yeast hydrolysate on expression of NOS in hypothalamic regions was investigated (Fig. 1). The staining intensities of the NOS

![Figure 1. Photomicrography of NOS expression in the PVN of the hypothalamus of normal diet-fed rats. A, control; B, BY-1 (0.1 g of yeast hydrolysate <10 kDa/kg body weight); C, BY-2 (1.0 g of yeast hydrolysate <10 kDa/kg body weight); D, AY-1 (0.1 g of 10–30 kDa yeast hydrolysate/kg body weight); E, AY-2 (1.0 g of 10–30 kDa yeast hydrolysate/kg body weight). The scale bar represents 100 μm.](image-url)
neurons in the PVN of the BY groups (oral administration of yeast hydrolysate <10 kDa) were significantly lower \((p < 0.05)\) than those of the AY groups (Figs 1 and 2). However, there was no significant difference between BY-1 and BY-2. The staining intensities of NOS neurons in the PVN of the BY group (oral administration of 10–30 kDa yeast hydrolysate) were higher than those of the BY groups and control (Fig. 1). Figure 2 shows the quantitative differences in the optical densities of NOS neurons in the hypothalamic regions from each group. The optical densities of NOS in the PVN of the AY groups were significantly higher than in the control and BY groups.

VIP, a 28-amino acid peptide, was originally isolated from the porcine gastrointestinal tract and subsequently found to be distributed widely in the peripheral and central nervous system (Said, 1991). Many studies have suggested that VIP functions as a neuromodulator in several regions of the central nervous system. Figure 3 shows the staining intensity of VIP immunoreactivity in the PVN and VMH of the hypothalamus. The staining intensities of VIP immunoreactivity in the PVN and VMH of BY groups were higher than those of the AY groups and control (Fig. 3). There were no differences in the VIP staining intensities in the PVN among the groups. However, the BY groups had higher VIP staining intensity in the VMH compared to control. Figure 4 shows the quantitative differences in the optical densities of VIP neurons in the hypothalamic regions of each group. The optical densities of VIP in the VMH of the BY groups were significantly higher than those of the AY groups \((p < 0.05)\). The optical densities of VIP in the PVN of the BY groups were slightly higher than that of the control group, but the differences were not significant.
DISCUSSION

The present study examined the effects of low and high molecular weight peptides of yeast hydrolysate on the body weight and plasma lipid levels in rats. The results showed that yeast hydrolysate <10 kDa reduced the body weight gain as well as TG and TC levels compared with the control. It is well known that dietary treatments such as energy restriction (Claery et al., 1987), fish oil diet (Parrish et al., 1990) and soy protein diet reduce body fat (Baba et al., 1992). In our previous study (Kim et al., 2004), it was demonstrated for the first time that intake of yeast hydrolysates, SR101, reduced the levels of plasma TG and the weight of epididymal and perirenal fat pads. At the whole body level, the hypotriacylglycerolaemic action of the yeast hydrolysates, especially hydrolysate <10 kDa, may be related to their effects on energy balance, as suggested by a positive correlation between the weight gain and the triacylglycerolaemia observed here (Tables 1 and 2). Previously, decreased body weight gain in rats was associated with diminished plasma triacylglycerol concentrations (Dobrian et al., 2000), and a positive correlation was observed in humans between the body fat mass and plasma TG (Nakanishi et al., 2000). However, the 10–30 kDa yeast hydrolysate did not exhibit a corresponding reduction in weight gain and TG levels.

The brain regulates energy homeostasis in response to signals from both adipose tissue and the gastrointestinal tract. The drive to eat and energy expenditure are adjusted so that, over time, the body weight remains stable. Weight regulation is a complex process involving the regulation of both food intake and energy output (Morley, 1987). A number of neurotransmitters and peptide hormones reduce food intake acutely but have minimal long term effects on body weight (Morley and Flood, 1987). For example, NOS inhibitors reduce food intake in rodents (Morley and Flood, 1992; 1994). NO is a biological messenger molecule that is synthesized in high levels in the brain and other mammalian tissues (Huang, 2000). The staining intensities of NOS neurons in the PVN of the BY groups were lower than those of the control and AY groups (Figs 1 and 2). Meanwhile, yeast hydrolysate fractions <10 kDa exhibited an NOS inhibitory activity, but fractions of 10–30 kDa yeast hydrolysate were activators of NOS.

Recently, Morley and Flood (1994) demonstrated that chronic administration of a NOS inhibitor can reduce weight by 10% in genetically obese (ob/ob) mice with an accompanying decrease in food intake. Squadrito et al. (1993) have found that obese Zucker rats also reduced food intake and body weight when treated with an NOS inhibitor. Morley et al. (1992) proposed that NOS increased in obese (ob/ob) mice. These results indicate that NO plays a major role in the hyperphagia of obese animals and that NO might be a physiological mediator in the mechanism controlling food intake. The results indicate that supplements of yeast hydrolysate <10 kDa reduced the expression of NOS in the PVN, which is known as a feeding center.

It has recently been reported that NO, which is a ubiquitous neurotransmitter, has functions related to VIP and regulates the release of VIP in the peripheral and central nervous system. In enteric nerve terminals, VIP and NO act in concert to cause relaxation or to inhibit intestinal contraction (Keef et al., 1994). In immunohistochemistry of the control, BY and AY rats, the VIP neurons were seen in all areas of the PVN and VMH in the hypothalamus (Fig. 3). In the PVN region, the treated groups, with the exception of the AY-2 group, had similar VIP staining intensities; however, the BY groups had a higher staining intensity than the others in the VMH region (Figs 3 and 4).

VIP and pituitary adenylate cyclase-activating polypeptide (PACAP) immunoreactive perikarya and nerve fibers were found in the hypothalamic paraventricular nucleus, VMH and lateral hypothalamic area (LHA), which are believed to regulate feeding in chickens (Peeters et al., 1998), also suggesting that brain VIP and PACAP may play a physiological role in appetite regulation. In mammals, limited conflicting information is available on the effect of VIP administration on food intake (Kulkosky et al., 1989; Woods et al., 1981), while

Figure 4. Optical densities of VIP immunoreactivity in the PVN and VMH of the hypothalamus of normal diet-fed rats. Values with differing superscripts are significantly different, p < 0.05. Values are mean ± SEM for 8 rats.
PACAP has been reported to inhibit food intake in mice (Morley et al., 1992). Since these peptides have been located by immunohistochemistry in several brain regions implicated in the control of feeding in goldfish (Tachibana et al., 2003), it is possible that these peptides are involved in the regulation of appetite in this species.

Although there is a possibility that the effect of appetite regulation may involve a reduction of body weight, it is not clear whether yeast hydrolysate, especially the fraction containing peptides <10 kDa, is suitable as a protein source for weight reduction. Here it is suggested that yeast hydrolysate, especially <10 kDa, might alter the expression of NOS and VIP neurons.

Further studies will investigate whether the yeast hydrolysate affects the hormone balance and will elucidate the principal components responsible for the anti-obesity effect.

In conclusion, the present results indicated that yeast hydrolysate <10 kDa reduced body weight gain and body fat in normal diet-fed rats and increased the lipid energy metabolism by altering the expression of NOS and VIP neurons. The above results suggest that yeast hydrolysate may have beneficial properties as a supplement with an anti-obesity effect for obese humans and/or those consuming normal diets. Therefore, the yeast hydrolysate <10 kDa may be recommended as a source for an anti-obesity functional food.

REFERENCES


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