State-Dependent Modulation of Feeding Behavior by Proopiomelanocortin-Derived β-Endorphin

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ABSTRACT: Feeding behavior can be divided into appetitive and consummatory phases, differing in neural substrates and effects of deprivation. Opioids play an important role in the appetitive aspects of feeding, but they also have acute stimulatory effects on food consumption. Because the opioid peptide β-endorphin is co-synthesized and released with melanocortins from proopiomelanocortin (POMC) neuronal terminals, we examined the physiological role of β-endorphin in feeding and energy homeostasis using a strain of mutant mice with a selective deficiency of β-endorphin. Male β-endorphin–deficient mice unexpectedly became obese with ad libitum access to rodent chow. Total body weight increased by 15% with a 50–100% increase in the mass of white fat. The mice were hyperphagic with a normal metabolic rate. Despite the absence of endogenous β-endorphin, the mutant mice did not differ from wild-type mice in their acute feeding responses to β-endorphin or neuropeptide Y administered intracerebroventricularly or naloxone administered intraperitoneally. Additional mice were studied using an operant behavioral paradigm to examine their acquisition of food reinforcers under increasing work demands. Food-deprived, β-endorphin–deficient male mice emitted the same number of lever presses under a progressive ratio schedule compared to wild-type mice. However, the mutant mice worked significantly less than did the wild-type mice for food reinforcers under nondeprived conditions. Controls for nonspecific effects on acquisition of conditioned learning, activity, satiety, and resistance to extinction revealed no genotype differences, supporting our interpretation that β-endorphin selectively affects a motivational component of reward behavior under nondeprived conditions. Therefore, we propose that β-endorphin may function in at least two primary modes to modulate feeding. In the appetitive phase, β-endorphin release increases the incentive value of food as a primary reinforcer. In contrast, it appears that endogenous β-endorphin may inhibit

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food consumption in parallel with melanocortins and that the orexigenic properties previously ascribed to it may actually be due to other classes of endogenous opioid peptides.

KEYWORDS: **β-endorphin; opioid peptides; operant conditioning; deprivation state; motivation; reinforcer; hyperphagia; knockout mice; metabolic rate; sexual dimorphism**

INTRODUCTION

The endogenous opioid system influences incentive-motivation in a number of different tests. Naloxone, a non-subtype–selective opiate receptor antagonist, reduces consumption of a variety of palatable foods and decreases operant responding for food reinforcers.[1,2] The endogenous opioid system also modulates self-administration of alcohol, benzodiazepines, psychostimulants, narcotics, and intracranial electrical self-stimulation.[3,4] In fact, a role for enkephalin in reward behavior was suggested soon after the first identification of an endogenous opioid.[5] Thus, a general role for the endogenous opioid system may be to enhance the incentive value of rewarding stimuli.

Many previous experiments have studied the role of the endogenous opioid system in reward-related behaviors by using subtype-selective opioid receptor antagonists. However, the endogenous opioid peptides interact relatively nonspecifically with the different opioid receptors, making it difficult to draw conclusions as to which endogenous opioids are involved in behaviors such as positive reinforcement.[6] β-Endorphin has nearly equal affinity for the mu and delta opioid receptor, and enkephalin preferentially binds to the delta receptor, although it also has physiologically relevant affinity for the mu receptor.[6] Agonists for all three opioid receptors can stimulate feeding to varying degrees, but agonists for the mu and delta receptor are thought to be intrinsically rewarding, whereas agonists for the kappa receptor have been shown to actually be aversive.[7] Thus, β-endorphin and enkephalin are the most likely opioid peptides to be involved in positively reinforced operant behavior. To test the function of one of these specific endogenous opioid peptides on energy homeostasis and feeding behavior in mice, we mutated the proopiomelanocortin (POMC) gene so that it does not express β-endorphin.[8]

UNCONDITIONED FEEDING BEHAVIOR IN β-ENDORPHIN-DEFICIENT MICE

To examine the effect of β-endorphin deficiency on baseline weight homeostasis, we analyzed sibling wild-type, heterozygous, and homozygous mice reared by heterozygous breeding pairs. Growth curves demonstrated that male β-$END^{-/-}$ mice weighed significantly more than male β-$END^{+/+}$ mice, starting at 4 weeks of age and continuing into adulthood (FIG. 1). By contrast, the weight of female β-$END^{-/-}$ mice differed only transiently from that of female β-$END^{+/+}$ mice between 4 and 8 weeks of age. β-$END^{+/-}$ mice did not differ from wild-type mice. Body length and the weight of various organs such as liver, spleen, kidney, heart, and gonads were not changed in either sex of β-$END^{-/-}$ mice. However, both the inguinal/gonadal and the

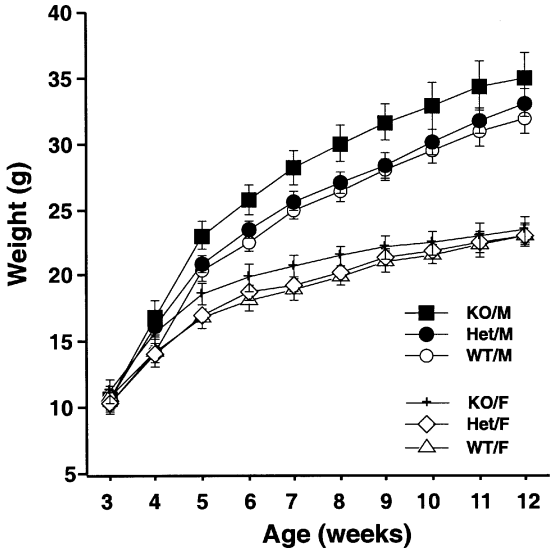

FIGURE 1. Growth curves of β-*END*$^{+/+}$ (WT), β-*END*$^{+/-}$ (Het), and β-*END*$^{-/-}$ (KO) male (M) and female (F) mutant mice reared by β-*END*$^{+/-}$ parents and fed a standard fat composition rodent chow (4% by weight) after weaning ($N = 11–29$ per group with the genotypes and sexes randomly distributed among a total of 19 separate litters with an average litter size of 6 pups).

retroperitoneal/perirenal white fat stores of male β-*END*$^{-/-}$ mice were twofold heavier than those of male β-*END*$^{+/+}$ mice, whereas intrascapular brown fat was not altered. Furthermore, male β-*END*$^{-/-}$ mice had 50% greater total body fat as measured by dual energy x-ray absorptiometry (DEXA)-scan, and histological examination suggested hypertrophy of adipocyte tissue.[9] The fat stores of female β-*END*$^{-/-}$ mice were not increased. An identical sexually dimorphic phenotype with equivalent or greater male-pattern obesity was observed in β-*END*$^{-/-}$ mice crossed onto either a 129S6/SV or an outbred Swiss albino background. These data indicate that development of obesity in β-endorphin–deficient mice is independent of the known genetic predisposition of C57BL/6 mice to gain excessive weight and adipose mass.

To determine the underlying metabolic reason for the increased weight and adiposity of the β-*END*$^{-/-}$ mice, we examined their food intake and basal metabolic rate. The average daily food intake of male β-*END*$^{-/-}$ mice was significantly increased compared to that of β-*END*$^{+/+}$ males. In contrast to the augmented food intake, no significant differences were noted between genotypes in their basal metabolic rate, as measured by oxygen consumption, respiratory quotient, basal core temperature, activity levels, or serum thyroxine levels.[9] Male β-*END*$^{-/-}$ mice gained more weight than did controls when fed a high-fat diet (9% vs. 5% fat content), but genotype differences paralleled changes on the regular chow (FIG. 2).

We examined whether the response of the β-*END*$^{-/-}$ mice to food intake stimulated by opioids or neuropeptide Y (NPY) was altered. β-Endorphin injected intra-

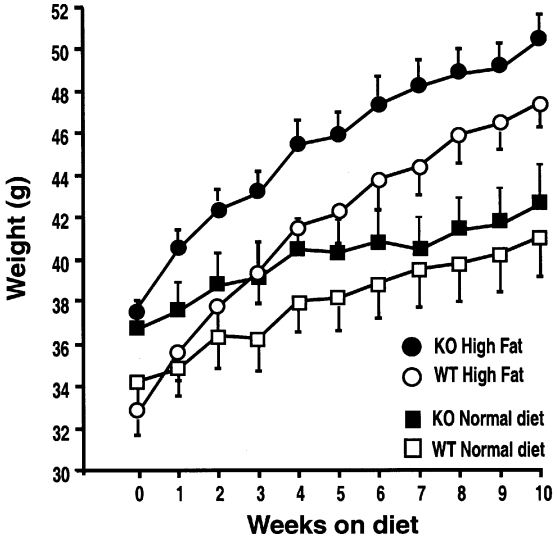

FIGURE 2. Weight gain of adult β-*END*$^{+/+}$ (WT) and β-*END*$^{-/-}$ (KO) male mice fed a normal-fat (4% by weight) and high-fat (9% by weight) chow (N = 6–8 per group).

cerebroventricularly stimulated an equivalent increase in food intake in both wild-type and β-*END*$^{-/-}$ mice.[9] Interestingly, the orexigenic effects of NPY were slightly increased in male β-*END*$^{-/-}$ mice, but unaltered in female β-*END*$^{-/-}$ mice, consistent with their normal weight and feeding behavior. However, NPY stimulation of food intake was inhibited equivalently by the nonselective opioid antagonist naloxone in both genotypes. Furthermore, naloxone also inhibited feeding to the same extent in previously food-restricted β-*END*$^{-/-}$ and wild-type mice. The retained actions of naloxone in β-endorphin–deficient mice do not appear to be explained by a compensatory increase in the expression of enkephalin or dynorphin in brain nuclei associated with feeding or reward behavior (FIG. 3).

OPERANT CONDITIONED FEEDING BEHAVIOR IN β-ENDORPHIN–DEFICIENT MICE

We tested for changes in the incentive value of rewarding stimuli by quantifying the reinforcing efficacy of food pellets using operant responding under a progressive ratio (PR) schedule, which requires additional bar presses of a defined number for each subsequent reinforcer.[10] PR schedules have been widely used to quantify the value an animal places on a commodity by measuring the effort it will expend to receive that reinforcer. In fact, this procedure has been successfully used to measure naloxone effects on motivation to obtain food reinforcers in rats and mice.[11–14] The incentive value of food varies with motivational states so that the neurobiological substrate underlying the instrumental behavior may be different in freely fed states from food-deprived states.[14,15] For example, the hedonic value of food may be the

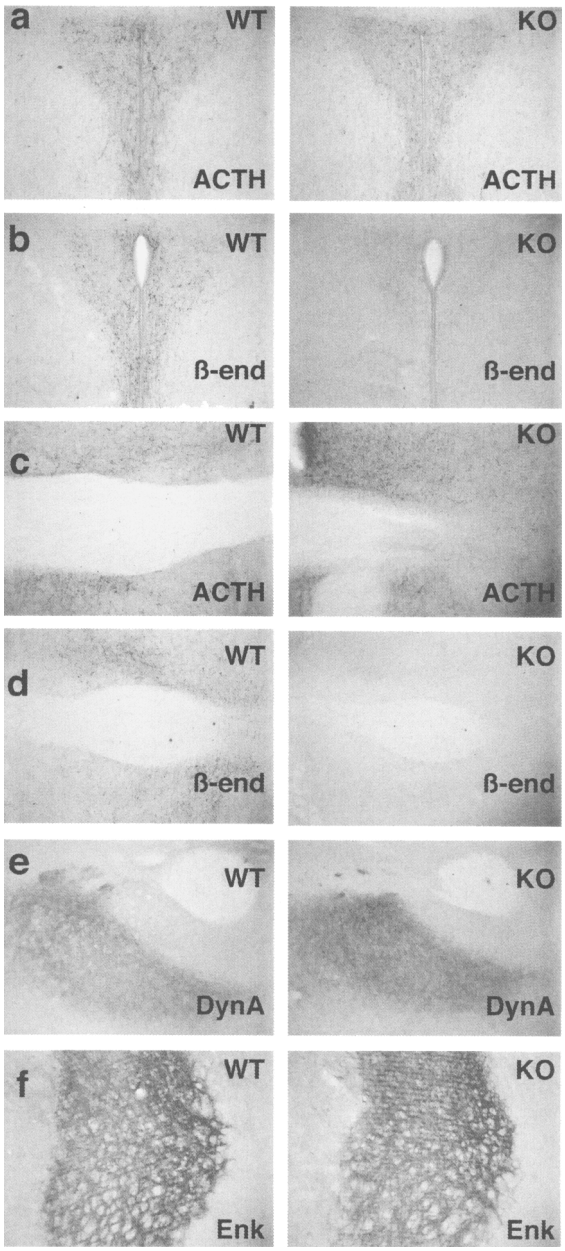

FIGURE 3. Expression of ACTH and opioid peptides in the brains of adult β-*END*$^{+/+}$ (WT) and β-*END*$^{-/-}$ (KO) male mice. ACTH, β-endorphin, dynorphin A, and Leu-enkephalin immunoreactive axons and terminals were detected with specific antisera and the ABC/diaminobenzidine technique on 50-µm coronal vibratome sections. (**a,b**) Paraventricular nucleus of the hypothalamus. (**c**,**d**) Bed nuclei of the stria terminalis dorsal and ventral to the anterior commissure. (**e**) Nucleus accumbens shell/ventral pallidum. (**f**) Globus pallidus. All digital photomicrographs were obtained at the identical magnification with a 10× objective and constant illumination.

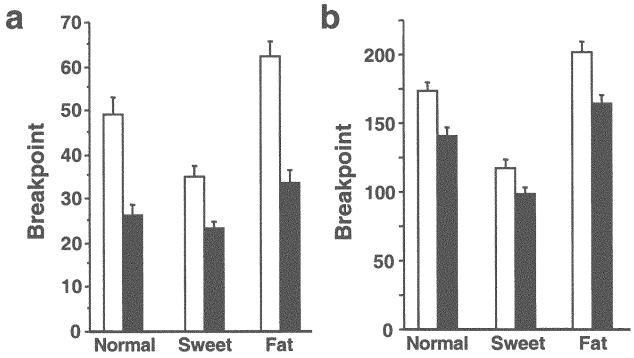

FIGURE 4. Operant responding for food reinforcers under a PR3 schedule and different states of food deprivation. (**a**) A significant difference was noted between genotypes in their breakpoints when the mice have *ad libitum* access to food in their home cages between operant sessions. (**b**) Food restriction to maintain mice at 75–80% of their normal body weight abolishes the breakpoint difference between the two genotypes, but it does not change the relative differences among the three types of food reinforcers. Note the large difference in scales on the *y*-axis between panels. β-$END^{+/+}$ (WT, *open bars*, $N = 9$) and β-$END^{-/-}$ (KO, *solid bars*, $N = 10$) male mice were used.

primary motivator in food-reinforced operant behavior under free-feeding conditions. Caloric imbalance would be the significant contributor to the incentive value of food reinforcers under food-deprived conditions.

β-$END^{+/+}$ and β-$END^{-/-}$ male mice were shaped initially to lever press for food reinforcers in an operant conditioning chamber under restricted feeding conditions. This training period consisted of a number of fixed ratio (FR) sessions first under an FR1, then under an FR5 before introducing the PR schedule. During the FR portion of the training period under restricted feeding conditions, the total number of reinforcers earned did not differ significantly between genotypes. Similarly, response rates on both active and inactive levers during the final FR5 session under *ad libitum* feeding conditions did not vary between genotypes. No main effect of genotype was detected by one-factor analysis of variance (ANOVA) on the active lever or inactive lever. Thus, all of the subjects performed at the same level before being introduced to the PR3 schedule.[16]

We compared the reinforcement efficacy between the genotypes by conducting PR3 sessions on *ad libitum* feeding subjects and measured the breakpoints (last ratio completed for a reinforcer) for each of three formulas of reinforcers: normal, sweet, and fat chow. Breakpoints were significantly higher for wild-type mice responding for all three formulas of reinforcers compared to the mutant genotype (FIG. 4a). Breakpoints of the β-$END^{-/-}$ male mice for the normal and fat chow reinforcers were only approximately half those of the wild-type mice. A main effect of chow formula and a chow formula by genotype interaction was also detected. However, separate repeated measures ANOVAs conducted on wild-type or β-endorphin–deficient mice detected main effects by chow formula for both genotypes. Thus, the rank order of preference was the same in all mice (fat chow > normal chow > sweet chow), but the level of behavior supported by these reinforcers differed between genotypes.

For mice in a nondeprived state, the endogenous opioids clearly modulated the efficacy of food reinforcers. However, in a food-deprived state induced by restricted access, the relative reinforcer efficacy was indistinguishable between the genotypes (FIG. 4b). Breakpoints did not differ significantly, and no main effect of genotype was detected under a PR3 schedule when mice were given restricted access to food. However, a main effect of chow formula was still detected. The rank order of preference was the same as in the *ad libitum* fed condition, but breakpoints were substantially higher under the food-restricted condition, and there was no genotype difference. This finding supports our argument that the difference shown in FIGURE 4a was not due to a motor deficit and suggests a state-dependent difference in motivation.

Differences in satiation between the genotypes under free-feeding conditions and a PR3 schedule could confound our interpretation of that data. Consequently, we examined operant behavior under an FR5, a relatively unchallenging and consistent schedule. The average duration of a PR3 session under *ad libitum* feeding conditions was approximately 1 h, so we used an FR5 schedule for 1 h to compare to the data gathered under a PR3 schedule during *ad libitum* feeding conditions. No main effect of genotype was detected for the number of pellets received under an FR5 schedule when mice were fed *ad libitum*, and there was no genotypic interaction by chow formula. However, a main effect of chow formula was detected, and the rank order of preference for the different reinforcers appeared to be the same as that found in the PR3 sessions, but there was no response difference between the genotypes under an FR5 schedule. In addition to response rates, we measured the number of pellets eaten during the 1-h FR5 sessions and compared these data to those of the PR3 sessions performed under *ad libitum* feeding conditions. During an FR5 schedule, mice from both genotypes ate significantly more reinforcers of all three formulas than they did during their PR3 testing. A significant main effect of schedule was detected by repeated measures ANOVA conducted on these data. This is unlikely to be an artifact of an increased workload under the PR3 schedule, because the average number of lever presses under the PR3 schedule was only roughly twice that under the FR5 (active lever presses by β-$END^{+/+}$ male mice for normal chow: PR3 = 497.6 ± 34.5 vs. FR5 = 253.6 ± 8.1). These data demonstrated that mice of both genotypes could eat significantly more pellets in 1 h than they ate during the PR3 sessions under *ad libitum* feeding conditions, suggesting that β-$END^{-/-}$ mice did not satiate earlier than did wild-type mice.[16]

Because a PR schedule uses an extinction criterion as an endpoint, we also examined the resistance to extinction by the two genotypes. Extinction sessions were conducted when the mice were being fed *ad libitum* using the PR3 schedule and a 15-minute limit to reach the next ratio, but with no reinforcers (PR3-EXT). The endpoint for extinction trials was determined by a *post-hoc* analysis and a criterion of 3 consecutive days that were not significantly different from each other (days 6–8). Extinction curves were generated using the data from days 1–6. These data confirmed that resistance to extinction was equivalent between genotypes and was not likely a contributing factor in the reduced breakpoints of the opioid mutant mice.[16]

While breakpoints under the PR3 schedule varied with the formula of reinforcer, we also noted an interaction between genotype and chow formula under the PR3 schedule in *ad libitum* fed mice. This interaction suggested that although the mutant genotypes varied their instrumental behavior for different formulas of reinforcers

with the same rank order, they did not vary their response to the same degree as did wild-type mice. Therefore, we determined if preference was altered in these subjects by testing consummatory behavior independently of instrumental behavior. Two-bottle free-choice experiments were conducted in the mice home cages using four concentrations of sucrose versus water and four concentrations of saccharin versus water.[16] Sucrose and saccharin were chosen because they allow a comparison of sweet compounds with and without caloric value and because an abundant number of studies have shown that opioid antagonists will decrease preference for these two compounds. Both genotypes increased their preference for the sucrose-containing bottle in an identical concentration-dependent pattern. Similarly, both genotypes had identical concentration-dependent changes in preference ratios for the saccharin-containing bottle. Basal water consumption in the home cage did not differ between the genotypes.

Mice normally balance their caloric intake with energy expenditure. When a highly palatable substance such as sucrose-flavored water is introduced into their diet, they will generally decrease their food intake to compensate for the extra caloric intake from sucrose. As shown above, the male β-*END*$^{-/-}$ mice are slightly hyperphagic at 1–4 months of age, so we determined if the caloric balance of intake was altered in the older mice used for the two-bottle, free-choice experiment. Food consumption in the home cages was measured while the two-bottle, free-choice experiments were conducted. Both genotypes decreased their food consumption with increasing concentrations of sucrose, and we detected no main effect of genotype on the amount of food eaten across four concentrations of sucrose, but a main effect of sucrose concentration was detected. Using saccharin, a noncaloric tastant, we also did not detect a main effect by genotype on the amount of food eaten across four concentrations. However, we detected a main effect of saccharin concentration on the amount of food eaten but no genotype by saccharin concentration interaction. The change in feeding during saccharin presentation was likely due to a change in the volume of liquid consumed, because the amount of food eaten did not decrease with higher concentrations of saccharin. Overall, it appeared that regulation of energy homeostasis was largely intact in the opioid mutant mice at older ages.[16]

CONCLUSIONS

Opioids have generally been shown to increase caloric intake, particularly the intake of highly palatable foods.[17–19] Similarities among the functions of opioids in the modulation of behaviors related to food intake, sexual activity, and drug abuse suggest a common action in the brain's reward circuits.[3] However, the role of each distinct endogenous opioid gene in these circuits is unknown. β-Endorphin is particularly intriguing because it is synthesized from a common prohormonal precursor together with the anorexigenic melanocortin peptides.[20,21] Our studies were designed to test the possible role of β-endorphin using a genetic approach that leaves intact the expression of all other opioid and opioid receptor genes.

Our data suggest that the effects of POMC-derived β-endorphin on feeding behavior are complex and dependent on sex steroid hormones, age of the animal, and deprivational state.[9,16] The development of obesity in young adult male β-endor-

phin–deficient mice indicates that endogenous β-endorphin may normally suppress, rather than stimulate feeding. Although this result at first appears paradoxical,[22] pharmacologic studies in the mutant mice showed a normal stimulatory effect of exogenous β-endorphin and an inhibitory effect of a nonspecific opioid agonist on food intake. It is possible that an unknown compensatory change has occurred during brain development in the β-endorphin–deficient mice, but studies to date indicate normal expression of enkephalin and dynorphin and the mu, delta, and kappa opioid receptors.[23,24] Furthermore, enkephalin-deficient mice do not develop obesity,[16] whereas mu-receptor–deficient mice exhibit a similar sexually dimorphic increase in body mass of males compared to the β-endorphin–deficient strain (Dr. B. Keiffer, personal communication). We therefore favor the interpretation that peptides other than β-endorphin are responsible for the physiological endogenous opioid tone in wild-type rodents that can be blocked with opioid receptor antagonists to decrease food intake.

By contrast, our data from the operant studies of lever pressing for food reinforcers strongly support the hypothesis that β-endorphin is an essential neurochemical component of the brain's reward circuitry. Intriguingly, the effect is only apparent under nondeprived conditions for food availability. Chronically food-restricted mice clearly have a strong incentive to work to obtain food reinforcers, but under these extreme conditions the absence of β-endorphin did not alter the motivated behavior. We also found that the absence of enkephalin and, in fact, the double mutation causing the absence of both β-endorphin and enkephalin produced essentially the same results on the operant tests for self-administration of food reinforcers.[16] This latter result is consistent with the idea that the two different opioid peptides converge on a common node in the neural circuitry underlying reward.

What are the implications of our data concerning the presumptive co-release of β-endorphin and melanocortin peptides from common POMC nerve terminals? One interpretation from an ethological perspective bears on the fact that typically a wild animal is more likely to be in caloric deficit than surfeit, and under this condition of deprivation the activity of POMC neurons and the expression of POMC are suppressed. Under the fortunate circumstances of locating an abundant source of calorie-dense food, POMC neuronal activity would increase. Although the acute actions of melanocortins, possibly in combination with those of β-endorphin, are to increase metabolic rate and decrease feeding, the longer-term actions of β-endorphin in the lateral hypothalamus, ventral tegmental area, and nucleus accumbens might be to strengthen the neural associations among the sensory, environmental, and nutritional qualities of the food cache to support the survival of the animal. Further experimental work is necessary to determine the relevant sites of action of β-endorphin and its interaction with the other opioid peptides and melanocortin peptides in this reward circuit.

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