The effect of melanocortin analogues on food intake and body weight change

Thesis by

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김 량 여

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ABSTRACT

The effect of melanocortin analogues on food intake and body weight change

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The central melanocortin system is involved in the regulation of food intake and body weight. Among the five melanocortin receptors subtypes, melanocortin 3 and 4 receptors (MC3R and MC4R) are expressed primarily in the brain and α-melanocyte stimulating hormone (α-MSH) has been shown to act on those receptors. To clarify its role on food intake and body weight, the effects of acute and chronic intracerebroventricular (I.C.V)

administration of several α-MSH analogues were investigated in mice. Acute I.C.V administration of α-MSH analogues, such as NDP-MSH, α-MSH-ND, [Gln^6]α-MSH-ND, [Lys^6]α-MSH-ND, which were substituted in the position of His^6 with Gln and Lys and Cyclic 16K-MSH to C57BL/6J mice showed a significant inhibition of food intake. However, the truncated form of [Gln^6]α-MSH-ND, which is lack of Nle^4-Asp^5, had no effect on food-intake. Chronic I.C.V administration of melanocortin analogues such as NDP-MSH, α-MSH-ND and [Gln^6]α-MSH-ND by osmotic minipumps significantly decreased both food intake and body weight for a period of 6 days, but the truncated form of [Gln^6]α-MSH-ND did show no significant effect. These findings support a role of melanocortin in the regulation of food intake and body weight control, and also demonstrated that the residues Nle^4 and Asp^5 of melanocortin peptides are important for their physiological function.

Key words: Melanocortin receptor, Peptide analogues, Obesity

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Ⅰ. Introduction

There are cumulative evidences indicating that several important players exist to regulate the food intake and energy homeostasis in central nervous system (CNS)[1,2,3]. One of the key factors is the leptin. Leptin is an adipose-derived hormone and acts to inhibit food intake and increase energy expenditure through

activation of CNS including the arcute nucleus (ARC)[4]. It has been reported that leptin stimulates catabolic pathway to decrease food intake and increase energy expenditure, whereas anabolic pathway is suppressed[5]. Among the catabolic mediators of leptin, the melanocortin system is well known for its importance in the regulation of energy homeostasis[6].

Over recent years, much attention has been drawn to the involvement of the melanocortins in the control of feeding behavior. Melanocortins are neuropeptides that are derived from the precursor peptide, pro-opiomelanocortin (POMC)[7,8]. This peptide mediates its effects through G-protein coupled receptors by stimulating adenylate cyclase. The melanocortin receptor (MCR) family consists of five receptor subtypes. The MC1R is expressed in melanocytes and is involved in skin pigmentation. The MC2R is the ACTH receptor and participates in the regulation of steroid production in the adrenal gland. The MC5R is expressed in many peripheral tissues and suggested to regulate pheromone production and thermal regulation[9,10,11]. On the other hand, MC3R and MC4R are expressed in the brain and are involved in energy homeostasis[12].

It has been reported that genetic disruption of MC4R caused

obesity in mice with hyperphagia[13]. In addition, targeted deletion of the MC3R in mice results in increased fat mass and reduced body mass despite the absence of hyperphagia and maintaining normal metabolic rates[14,15]. It was suggested that MC4R regulates food intake and energy expenditure, whereas MC3R influences feeding efficiency, indicating that MC3R and MC4R serve non-redundant roles in the regulation of energy homeostasis. Therefore, MC4R/MC3R selective agonists have been considered as potential candidates for treatment of obesity[16,17,18].

Previously, our groups analyzed the several different α-MSH analogues on stimulation of MC3R and MC4R using a CRE-mediated reporter gene transcription activity assay[19]. In this study, the effects of acute and chronic intracerebroventricular (I.C.V) administration of several α-MSH analogues were investigated to clarify their role on food intake and body weight change.

Ⅱ. Materials and Methods

1. Animals preparation

Male C57BL/6J mice weighing 20-30g (7-9 weeks old) were maintained into the cages with free access for feed and water under 12hr light cycle and 12 hr dark cycle (lights on at 8hr) with controlled temperature (21℃-23℃).

2. Analysis of food intake after intracerebroventricular (I.C.V) administration of melanocortin analogues

One-week before the administration of drugs by I.C.V infusion, mice were housed individually. Before I.C.V administration, mice were fasted for 24hr. Melanocortin peptides as described in Table 1 were prepared with concentration of 3nmole in 5$\mu\ell$ volume for injection. Melanocortin peptides or saline were slowly administered into third ventricular in mice by using Hamilton syringe (26 gauge needle)[18,20]. After injection, mice were returned to their home cages containing a premeasured quantity of food pellets. Peptide administration was performed at the beginning of the light phase and food intake was measured at 2, 4, 6, 8, 10 and 12hr after administration of peptides. Food intake measurement for a given

peptide at time point was analyzed by ANOVA followed by Dunnett test (*p<0.05, **P<0.01).

Table 1. Amino acid sequence of melanocortin analogues used in this study

	Amino acids												
	1	2	3	4	5	6	7	8	9	10	11	12	13
α-MSH	Ser	Tyr	Ser	Met	Glu	His	Phe	Arg	Trp	Gly	Lys	Pro	Val
NDP-MSH	Ser	Tyr	Ser	Nle	Glu	His	D-Phe	Arg	Trp	Gly	Lys	Pro	Val
α-MSH-ND				Nle	Asp	His	D-Phe	Arg	Trp	Lys			
[Gln6] α-MSH-ND				Nle	Asp	Gln	D-Phe	Arg	Trp	Lys			
[Lys6] α-MSH-ND				Nle	Asp	Lys	D-Phe	Arg	Trp	Lys			
[Gln6] α-MSH-ND(6-10)						Gln	D-Phe	Arg	Trp	Lys			
Cyclic 16K-MSH					Cys	His	D-Phe	Arg	Cys	Lys			
JKC-363	Cyclic												
	Mpr	Glu	His	D-Nal	Asp	Trp	Gly	Cys	Pro	Pro	Lys		

3. Analysis of food intake and body weight after intracerebroventricular (I.C.V) administration of melanocortin analogues

One-week before the administration of drugs by I.C.V infusion, mice were housed into the cages. The mouse was anaesthetized by intraperitoneal (i.p.) injection of ketamine (130 mg/kg) and xylazine (8.8 mg/kg). A brain infusion cannula (Alzet Brain Infusion Kit, Alza Co., Palo Alto, CA, USA) was stereotaxically implanted into the right lateral ventricle (coordinates: 0.3 mm caudal, 1 mm lateral from bregma, and depth 2.5 mm) and it was fixed to the skull with dental cement[21,22,23]. The cannula was connected by tubing to the flow moderator of micro osmotic pump (Alzet Model 1007D, flow rate of 0.5 $\mu\ell$/h). Osmotic pump were inserted into the subcutaneous pocket. Prior to the infusions, pumps had been filled with either melanocortin peptides (10 nmol/day) or saline and placed for overnight[21,24]. Therefore, infusion of melanocortin analogues or saline started immediately after surgery. Mouse were allowed to recover from anaesthesia and returned their home cages containing a premeasured quantity of food pellets. Food intake and body weight were recorded 2hr after

lights on and 2hr before lights off for the 6 days of infusion period[23,25]. After 6 days of infusion, the animals were sacrificed. Data of food intake and body weight for a given peptide were analyzed by ANOVA followed by Dunnett test (*p<0.05, **P<0.01).

Ⅲ. Results

1. Effect of I.C.V administration of the melanocortin analogues on food intake and body weight.

Mice were fasted for 24hr before I.C.V administration of α-MSH analogues. Different melanocortin analougues [NDP-MSH, α-MSH-ND, [Gln^6]α-MSH-ND, [Lys^6]α-MSH-ND, cyclic16K-MSH and [Gln^6]α-MSH-ND(6-10): Table 1] were administered and their effect on food intake inhibition was analyzed *in vivo*. Food intake was measured at 2, 4, 6, 8, 10 and 12hr after administration of peptides. Fig. 1 showed the time-course of cumulative food intake over 12hr (A) period and cumulative food intake for 10hr (B). NDP-MSH and α-MSH-ND significantly inhibited food intake by up to 65-70% as compared to the saline-administered control over 10hr period (P<0.01). Other peptides, such as α-MSH, [Gln^6]α-MSH-ND, [Lys^6]α-MSH-ND and cyclic16K-MSH also significantly inhibited food intake by 36-50% over 10hr period (* P<0.05, n=10) after administration. However, the truncated form of [Gln^6]α-MSH-ND showed no significant effect on food intake (Fig. 1). Therefore, NDP-MSH and α-MSH-ND were the potent peptides for inhibition of food intake, mean while [Gln^6]α-MSH-ND, [Lys^6]α-**

MSH-ND and cyclic16K-MSH also showed significant inhibition of food intake.

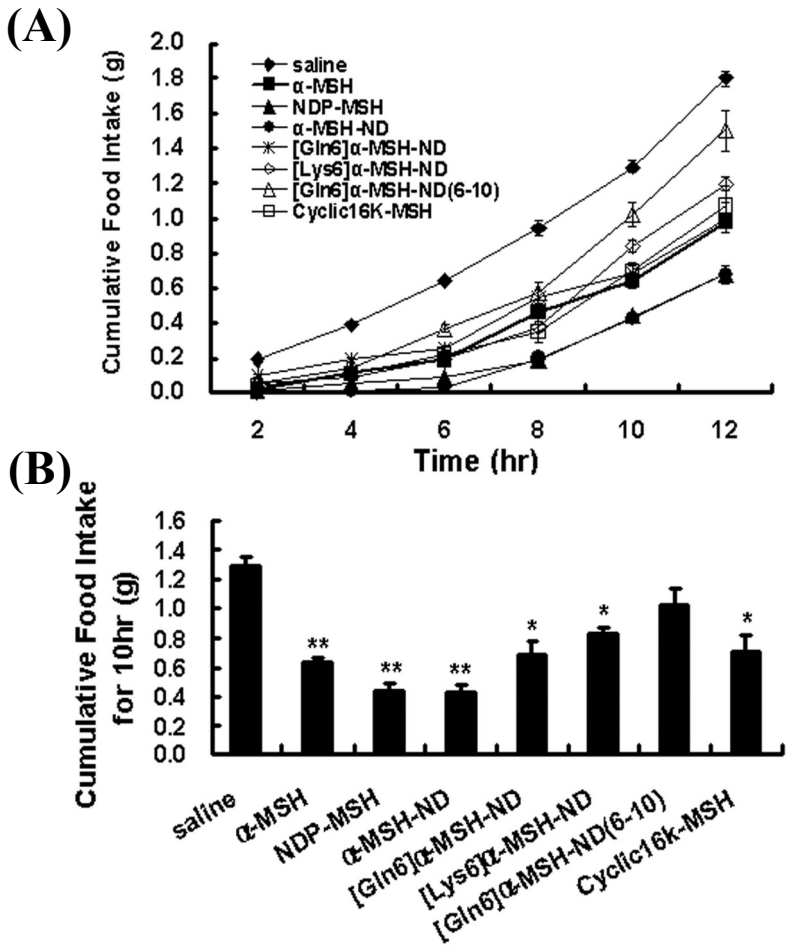

Fig. 1. Inhibition of food intake by acute I.C.V administration of several melanocortin analogues. α-MSH, NDP-MSH, α-MSH-ND, [Gln^6]α-MSH-ND, [Lys^6]α-MSH-ND and cyclic16k-MSH (3nmole) produced the significant inhibition of food-intake whereas the truncated form of [Gln^6]α-MSH-ND (3nmole) had no significant effect on food intake. (A) cumulative time course food intake over 12hr period. (B) cumulative food intake for 10hr. All value are expressed as mean±SEM, n=10 per each group. Data were analyzed by ANOVA followed by Dunnett test for individual comparisons. (*$p<0.05$, **$p<0.01$ vs. saline)

2. Effect of chronic I.C.V administration of the melanocortin analogues on food intake and body weight.

The chronic effects of melanocortin analogues were investigated on food intake and body weight change by osmotic minipumps infusion system. Osmotic minipumps, which were connected to I.C.V by cannula, were used for continuous 6 days of infusion for α-MSH analogues such as NDP-MSH, [Gln^6]α-MSH-ND, α-MSH-ND and [Gln^6]α-MSH-ND(6-10) in mice. The results on cumulative food intake were shown in Fig. 2A and those on body weight in Fig. 2B. Both the dose 3nmol/day and 10nmol/day of NDP-MSH decreased the food intake and body weight during 6 days of chronic infusion compared with those of the saline-treated group. NDP-MSH at a dose 10nmol/day was more efficient in the inhibition of food intake and body weight loss than both effects of NDP-MSH at a dose 3nmol/day (Fig. 2). These results indicated that chronic I.C.V administration of NDP-MSH in mice decreased food intake and body weight in a dose-dependent manner (Fig. 2).

Chronic I.C.V administration of α-MSH analogues, such as [Gln^6]α-MSH-ND, α-MSH-ND at a dose 10nmol/day resulted in a greater body weight loss than control. Fig. 3 showed the cumulative

food intake (Fig. 3A) and body weight gain (Fig. 3B) during infusion of saline or α-MSH analogues. In NDP-MSH and [Gln^6]α-MSH-ND treated mice, loss of body weight was observed during the first 2 days of treatment. Their cumulative body weight loss remained significantly until third day. However, [Gln^6]α-MSH-ND(6-10)-treated mice had no significant effect on food intake and body weight.

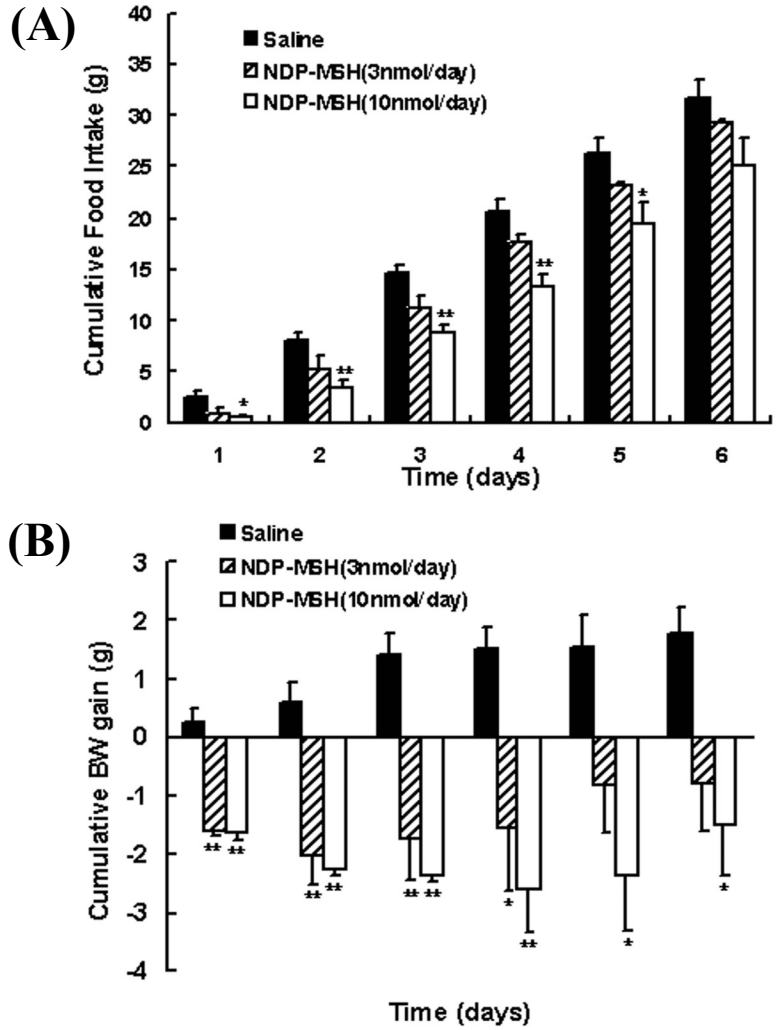

Fig. 2. Dose-dependent change of food intake and body weight by chronic I.C.V administration of NDP-MSH. The doses of 3nmol/day and 10nmol/day of NDP-MSH decreased the body weight and food intake during 6 days of chronic infusion compared with those of the saline. (A) cumulative food intake over 6 days period. (B) cumulative body weight gain over 6 days period. All value are expressed as mean±SEM, n=5 per each group. Data were analyzed by mean of ANOVA followed by Dunnett test for individual comparisons. (*P<0.05, **P<0.01 vs. saline)

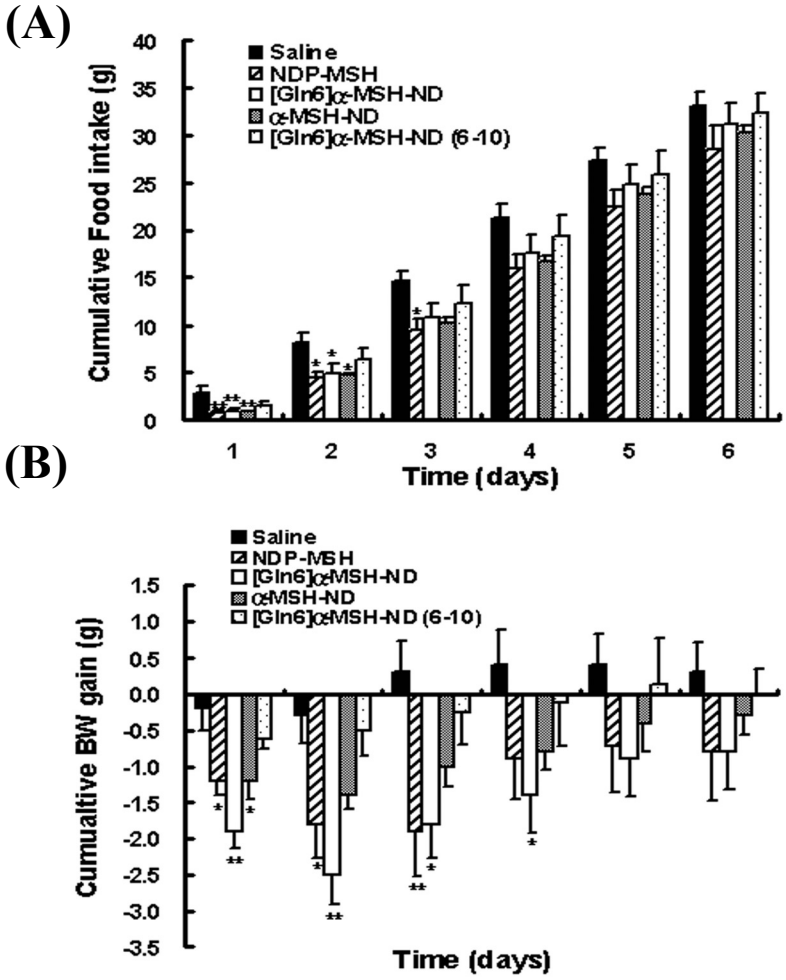

Fig. 3. Inhibition of food intake and body weight by chronic I.C.V administration of melanocortin analogues. NDP-MSH, [Gln^6]α-MSH-ND, α-MSH-ND (10nmol/day) produced a significant inhibition on food intake and body weight gain. (A) cumulative food intake over 6 days of period. (B) cumulative body weight gain over 6 days of period. All value are expressed as mean±SEM, n=5 per each group. Data were analyzed by mean of ANOVA followed by Dunnett test for individual comparisons. (*P<0.05, **P<0.01 vs. saline)

3. Effect of JKC-363 on NDP-MSH or [Gln^6]α-MSH-ND- induced food-intake.

Previously, several different α-MSH analogues were analyzed on stimulation of MC3R and MC4R using a CRE-mediated reporter gene assay. In the reporter gene assay, [Gln^6]α-MSH-ND was shown to be the most selective α-MSH agonist on MC4R[19]. In the present study, the effect of JKC-363 (cyclic [Mpr, D-Nal, Cys, Asp-NH_2]- α-MSH 11-22), a selective MC4R antagonist, was analyzed on the NDP-MSH and [Gln^6]α-MSH-ND-induced decrease of food intake[27].

The administration of JKC-363 efficiently blocked the decrease in food intake induced by [Gln^6]α-MSH-ND, showing an increase of cumulative food intake at 2hr as compared with that induced by [Gln^6]α-MSH-ND alone. The effect of JKC-363 to block the decrease in food intake was more efficient when JKC-363 was administered with [Gln^6]α-MSH-ND than with NDP-MSH (Fig. 4B).

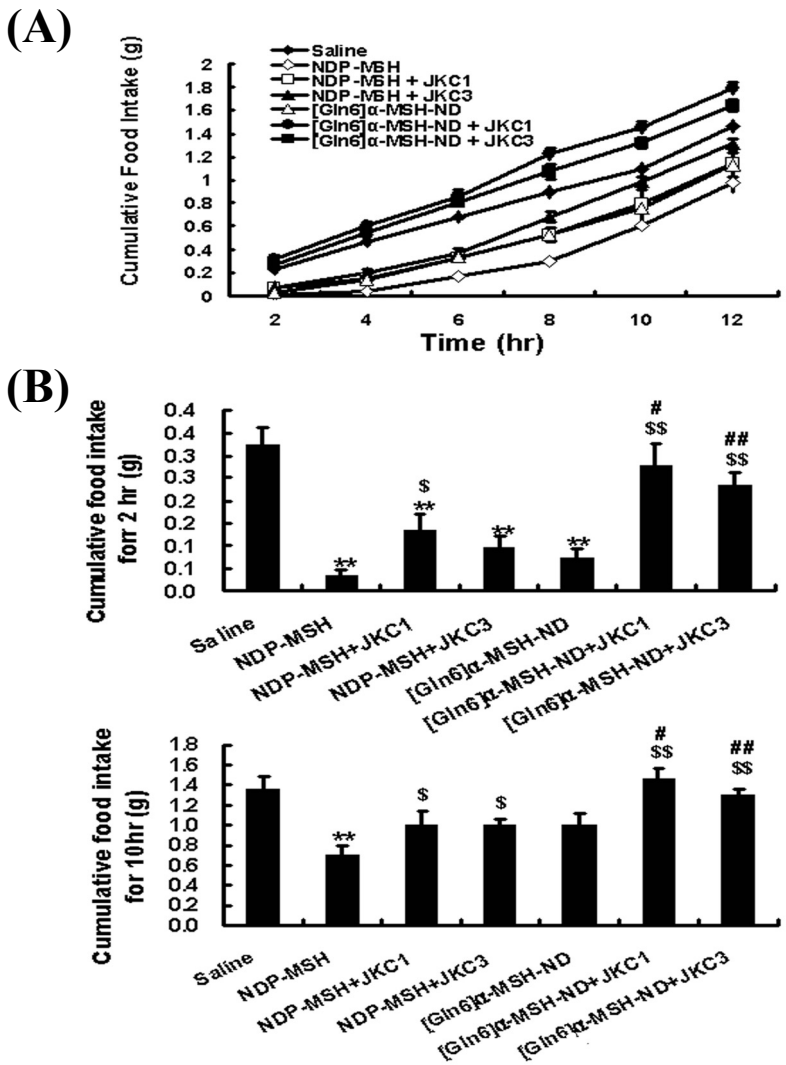

Fig. 4. Effect of JKC 363 on food intake induced by α-MSH analogues. NDP-MSH or [Gln6]α-MSH-ND was administered together with MC4R-selective antagonist JKC363 (each 1 and 3 nmole). (a) cumulative food intake over 12 hr period. (b) cumulative food intake for 2hr and 10hr. All value are expressed as mean ±SEM, n=9 per each group. Data were analyzed by ANOVA followed by Dunnett test for individual comparisons. (*P<0.05, **P<0.01 vs. saline) ($P<0.05, $$P<0.01 vs [Gln6]α-MSH-ND or NDP-MSH (paired t test)) (#P<0.05, ##P<0.01 vs. NDP-MSH+JKC363 (unpaired t test)

Ⅳ. Discussion

The hypothalamic melanocortin system plays a critical role in appetite and body weight regulation[7,8]. Recently, many agonists of MC3R or MC4R have been synthesized and analyzed in the view of receptor selectivity and biological activity to evolve as an anti-obesity medicine[27,28,29,30].

Previously, the differential regulation of cAMP-mediated gene transcription and ligand selectivity by MC3R and MC4R with several melanocortin analogues has been reported *in vitro*[19]. In the present study, the acute and chronic effects of several melanocortin analogues (Table 1) on food intake and body weight change were investigated *in vivo*.

Acute I.C.V administration of melanocortin analogues to C57BL/6J mice showed that NDP-MSH and α-MSH-ND were the potent peptides on the inhibition of food intake. The other peptides, such as α-MSH, [Gln^6]α-MSH-ND, [Lys^6]α-MSH-ND and cyclic 16K-MSH, also showed a substantial suppression of cumulative food intake. However, the truncated form of [Gln^6]α-MSH-ND had no effect. These observations *in vivo* showed a strong consistency with reporter gene assay, which was assessed in terms of G protein

coupling efficiency by these analogues *in vitro*[19]. In the reporter gene assay, [Gln^6]α-MSH-ND was the most selective melanocortin analogue for MC4R, whereas the truncated form of [Gln^6]α-MSH-ND showed loss of reporter gene activation with MC4R and MC3R[19]. These results also suggested the structural importance of amino acid sequence of melanocortin analougues for their physiological function *in vivo*[26]. It has been reported that the core-sequence of α-MSH analogues (His-D-Phe-Arg-Trp) is critical for improving the potency and receptor selectivity of the ligand at the MC3R and MC4R[26,31]. Recently, it was also reported that the His^6 position is the most critical position for increasing MC3R versus MC4R selectivity, therefore the modification at the His^6 position in the tetrapeptide (His-D-Phe-Arg-Trp) may be utilized to improve MC4R selectivity[26]. [Gln^6]α-MSH-ND which has a modified core-sequence (Gln-D-Phe-Arg-Trp) showed the significant inhibitory effect on food intake and displayed comparable potency to α-MSH-ND for MC4R. However, the truncated form of [Gln^6]α-MSH-ND which is lack of Nle^4 and Asp^5 did not show any significant effect *in vivo*[19]. These data indicate that the residues in the position 4 and 5

(Nle^4-Asp^5) of melanocortin peptides are also important for the physiological functions.

The chronic infusion of melanocortin analogues such as NDP-MSH, [Gln^6]α-MSH-ND and α-MSH-ND induced a significant decrease in food intake and body weight. Significant body weight loss was also observed in NDP-MSH and [Gln^6]α-MSH-ND treated mice until the third day after osmotic pump infusion. These data suggested that a chronic administration of melanocortin analogues decrease food intake with concomitant decrease in body weight. The increase in food consumption at day 4 of treatment was accompanied by a plateauing of the weight curve to the level of the control mice. It might be interpreted as that NDP-MSH and [Gln^6]α-MSH-ND had reduced but not totally lost effects on food intake until the third day after treatment. In contrast, the truncated form of [Gln^6]α-MSH-ND did not show significant effect neither on food-intake or body weight change. These results demonstrate that the residues Nle^4 and Asp^5 of melanocortin peptides are important for physiological functions which regulate on food intake and body weight. Therefore, other approaches such as changing the sequence of the residues in position 4 and 5 are

needed to elucidate the physiological roles of amino acid sequence of melanocortin analogues. Taken together, these results indicate that the MCR stimulation by prolonged I.C.V administration of α-MSH analogues in mice induced major suppression of food intake and weight loss. Further studies are needed to clarify the chronic effect of α-MSH analogues according to the different periods of treatment.

Another important finding of this study is that [Gln^6]α-MSH-ND can act as a selective MC 4R agonist *in vivo* as suggested also *in vitro*, previously[19]. JKC-363 blocked the suppressive effect of [Gln^6]α-MSH-ND and NDP-MSH on food intake (Fig. 3). JKC-363 is known as a potent and selective MC4R antagonist and is more potent than HS014, which is another MC4R antagonist[31,32]. These results may suggest that [Gln^6]α-MSH-ND can be a MC4R-selective melanocortin analogue among the different melanocortin analogues that we have investigated.

In conclusion, acute and chronic I.C.V administration of several melanocortin analogues efficiently decreased the food intake and body weight in mice. These results suggest that the residues Nle^4 and Asp^5 of melanocortin peptides are important for

determination of the role of potency on food intake and body weight regulation. Identification of potential target of MC4R agonists is currently undertaking to understand the signalings mediated by melanocortin in the feeding circuitry in central nervous system.

Ⅴ. Conclusion

In the present study, the effects on food intake and body weight change by acute and chronic I.C.V administration of melanocortin analogues were investigated. These results have led me to the following conclusions.

1. **Acute I.C.V administration of different melanocortin analogues showed that NDP-MSH and α-MSH-ND were the most efficient peptides in inhibition of food-intake and other peptides, such as α-MSH, [Gln^6]α-MSH-ND, [Lys^6]α-MSH-ND and cyclic 16k-MSH, also significantly inhibited food intake whereas [Gln^6]α-MSH-ND(6-10), the truncated form of [Gln^6]α-MSH-ND had no effect.**
2. **Chronic I.C.V administration of melanocortin analogues, such as NDP-MSH, α-MSH-ND and [Gln^6]α-MSH-ND significantly decreased both food intake and body weight, but [Gln^6]α-MSH-ND(6-10), the truncated form of [Gln^6]α-MSH-ND did not show significant effect on inhibition of food-intake and body weight for a period of 6 days.**

3. **Administration of JKC-363, a selective MC4R-specific antagonist in combination with [Gln^6]α-MSH-ND specifically inhibited the decrease in food intake induced by [Gln^6]α-MSH-ND, suggesting that this peptide can be a selective MC4R agonist.**

Taken together, these results suggest that the residues Nle^4 and Asp^5 of melanocortin analogues are participated in the activation of receptor and physiological functions in food intake and body weight regulation.

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국문요약

멜라노코틴 유도체의 식욕조절과 체중 변화에 미치는 효과

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멜라노코틴의 일종인 α-melanocyte stimulating hormone (α-MSH)은 전구물질인 pro-opiomelanocortin (POMC)의 산물로서 13개의 아미노산으로 구성된 펩타이드 호르몬이다. 멜라노코틴과 결합하는 5개의 멜라노코틴 수용체중에서 멜라노코틴 제 3수용체(MC3R)와 멜라노코틴 제 4수용체(MC4R)는 비만의 병리에 매우 중요한 작용

을 하는 것으로 알려져 있다. 이에 MC3R과 MC4R을 중심으로 멜라노코틴 유도체들이 시상하부에서의 식욕조절에 미치는 영향을 관찰하기 위하여, 본 연구에서는 α-MSH의 Phe^{6}을 D-Phe^{6}로 아미노산 잔기 치환을 통해 제작된 NDP-MSH, α-MSH-ND와 α-MSH-ND의 6번째 His^{6}을 Gln과 Lys으로 치환한 [Gln^{6}]α-MSH-ND, [Lys^{6}]α-MSH-ND 그리고 Cyclic16k-MSH 등의 유도체들을 단기적으로 뇌실 주입하였던 바 마우스의 섭식이 감소됨을 관찰하였다. 그러나 [Gln^{6}]α-MSH-ND의 절단형인 [Gln^{6}]α-MSH-ND(6-10)인 경우 섭식이 크게 영향을 받지 않았으며, MC4R에 선택적인 길항제인 JKC-363을 단기 뇌실 주입하였을 때 [Gln^{6}]α-MSH-ND에 의해 감소된 섭식이 효과적으로 증가하는 것으로 보아 [Gln^{6}]α-MSH-ND이 MC4R의 선택적인 agonist임을 확인하였다. 결론적으로 I.C.V로 주입된 멜라노코틴 유도체들은 MC4R/MC3R 표현 세포주에서 관찰된 것과 유사한 활성도를 나타냄을 관찰할 수 있었다. 또한 멜라노코틴 유도체들을 6일 동안 장기적으로 뇌실 주입한 결과 NDP-MSH, α-MSH-ND, [Gln^{6}]α-MSH-ND을 투여한 마우스는 섭식과 몸무게 감소가 관찰되었다. 반면 [Gln^{6}]α-MSH-ND(6-10)에 의해서는 단기 뇌실 주입시 관찰된 것과 같이 섭식 및 몸무게에 크게 영향을 주지 않음을 관찰할 수 있었다. 이것은 멜라노코틴 유도체의 4, 5번째 잔기가 식욕섭취와 몸무게 조절에 중요한 역할을 하고 있음을 보여주

고 있다. 흥미롭게도 식욕억제 및 체중감소효과를 보였던 유도체들은 투여 제 4일째부터 억제 효과가 감소됨을 알 수 있었다. 멜라노코틴 유도체들의 투여 제 4일 이후 식욕억제 및 체중감소효과가 감소된 것에 관하여 향후 장기 투여에 따른 감소효과에 대한 연구가 더 필요한 것으로 사료된다.

핵심되는 말 : 멜라노코틴, 유도체, 섭식, 몸무게