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# Melanin-concentrating hormone enhances sucrose Intake

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【 １２２ 】

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【 学位論文題目 】

Melanin-Concentrating Hormone Enhances Sucrose Intake
（メラニン凝集性ホルモンとショ糖摂取との関連）

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Melanin-concentrating hormone (MCH) is a cyclic neuropeptide originally isolated from teleost fish, in which it regulates skin pigmentation. MCH is expressed in the central nervous system predominantly in neurons of the lateral hypothalamus (LH) and of the zona incerta (ZI), the brain sites responsible for feeding and drinking behaviors. MCH has been confirmed to be involved in regulating food intake and energy balance through a series of experiments, including intracerebroventricular (i.c.v) injection of MCH or MCH receptor blockers, and genetic manipulation of MCH and its receptor.

MCH was reported to stimulate water intake independent of food intake. Diabetic animals such as db/db mice and OLETF rats were reported to show greater neural and behavioral responses to sugars than lean, non-diabetic controls. The purpose of this study is to investigate details of the dipsogenic response of MCH, with special emphasis on sweet taste solution.

**Research design and Method**

**Drugs and Animals.** Experimental drugs were Human/rat MCH (hMCH), salmon MCH (sMCH), agouti-related protein (83-132) (AgRP). Angiotensin II (ANG II) and its specific antagonist, [Sar1, Ala8] ANG II (saralasin) and [Sar1, Val5, Ala8] ANG II. For the bottle choice tests, sucrose, D-glucose, saccharin and NaCl solution were used. Adult male Sprague-Dawley rats weighing 300-450g were implanted with a cannula stereotaxically placed in the intracerebroventricular for the experimental drug injection.

**Feeding experiments.** Cumulative food and water intake and body weight were measured for four hours after the drug or control saline injection. This experiment was also performed in the absence of food (n=5-10 per group).

**The bottle-choice test.** The bottle-choice tests were conducted giving a choice between sucrose, NaCl and water for hMCH, sMCH and ANG II in the absence of food and between sucrose and water for hMCH, sMCH and AgRP in the presence and absence of food (n=5-10 per group).Then preferences for a series of concentrations of sucrose and glucose solution (0.0026M-0.26M) were investigated in hMCH-, sMCH- and saline- treated rats in the absence of food (n=5-12 per group). Moreover, the intake of saccharin solution and water were measured in the absence of food (n=5-9 per group).

**RNA isolation and Real-Time RT-PCR.** Rats were provided with sucrose and tap water for 5 days with ad libitum access to food. The RNA isolation was performed for the real time RT- PCR study. Quantification of mRNA levels was performed with SYBR-green chemistry (Qiagen) using a one-step RT-PCR reaction on an ABI PRISM 7700 Sequence Detection System. The rat G3PDH gene was used as an internal control.

**Statistical analysis.** Results were analyzed by one-way ANOVA, and the Dunnett comparisons were employed to examine significant alterations. Values of $p<0.05$ were considered statistically significant. Pearson's correlation coefficient was calculated in the real time RT-PCR study. Correlation of $p<0.05$ (2-tailed) was considered statistically significant.

**RESULTS**

**Effect of i.c.v. injection of hMCH and sMCH on water intake in the presence and also absence of food.** The i.c.v. injection of hMCH and sMCH increased food as well as water intake as previously reported. The hMCH produced a significant increase in water intake only at the highest dose (6.0 nmol), whereas

sMCH significantly stimulated water intake from 1.5nmol. The 6.0 nmol of hMCH increased water intake by 1.6 times and sMCH more than twofold as compared with saline.

**Effect of i.c.v. injection of hMCH, sMCH and ANG II on water, NaCl and sucrose solution intake.** hMCH- or sMCH- induced water intake was not attenuated by the pretreatment with ANG II antagonist saralasin or [Ala1, Val5, Ala8] ANG II. The hMCH and sMCH significantly stimulated sucrose intake more than twofold as compared with saline, but failed to stimulate water and NaCl solution intake. ANG II, on the other hand, not only increased sucrose but also water and NaCl solution intake.

**Effect of i.c.v. injection of hMCH, sMCH and AgRP on sucrose intake.** Although hMCH- and sMCH- treated rats significantly drunk 0.26M sucrose, these two groups of animals still consumed six to eight times more food relative to saline. In contrast, AgRP significantly increased food intake, but not sucrose intake, although it increased sucrose intake in the absence of food.

**Effect of i.c.v. injection of hMCH and sMCH on sucrose, glucose and saccharin intake.** The intake of sucrose, glucose and saccharin solutions and water were examined in hMCH- and sMCH- treated rats in the absence of food. MCH-treated rats significantly increased the ingestion of sucrose and glucose solution compared to saline. There was no significant difference in saccharin intake between groups.

**The hypothalamic mRNA levels of MCH and MCHR1.** Hypothalamic MCH and MCHR1 mRNA levels increased as the concentration of sucrose increased during the course of 5-days sucrose intake. A significant correlation was observed between sucrose intake at various concentrations and MCH mRNA levels ($r = 0.57$, $p<0.006$). MCHR1 mRNA levels were also strongly correlated with sucrose intake ($r = 0.66$, $p<0.001$).

## DISCUSSION

In this study we demonstrated that i.c.v. injection of hMCH and sMCH acutely increase food and water intake in rats as reported in previous studies. We also demonstrated that hMCH and sMCH increase water intake independent of food intake, which was more obvious in sMCH than hMCH. A high degree of amino acid sequence homology was found between sMCH and hMCH. In the minimal active fragment, the substitution of $Leu^9$ by $Val^9$ is the only difference between hMCH and sMCH and structure-activity studies demonstrated that both hMCH and sMCH activate MCH1R in rats similarly although sMCH activates MCH2R with a reduced potency. The potential differences in the human and salmon MCH actions on water intake need to be further examined.

ANG II is a potent dipsogenic hormone that causes thirst and salt appetite for the maintenance of blood pressure and fluid balance. Our findings that MCH-induced water intake was not suppressed by ANG II antagonist and MCH did not cause salt appetite, suggest that MCH affects hypothalamic neuronal circuits independent from ANG II system.

In this study, we demonstrated for the first time that hMCH and sMCH increased sweet caloric-solution intake, even at the lower concentrations than those normal rats prefer. It is known that MCH gene expression in the LH of the Ay/a mice that overexpress agouti protein is increased. AgRP has an especially potent influence on the intake of a palatable diet. We thus compared the acute effect of hMCH, sMCH and AgRP on sucrose solution and food intake, and found that hMCH and sMCH

increased both sucrose and food intake, whereas AgRP only increased food intake. Importantly, when food was not present AgRP dramatically increased sucrose intake. Similar to our study, Olszewski and his colleague have reported that injection of AgRP into the paraventricular nucleus (PVN) increased food intake but not sucrose solution intake. When rats were given a choice between food and a 10% (0.3M) sucrose solution, $\mu$-opioid receptor agonist DAMGO ingested more sucrose solution than food. These observations are consistent with the notion that AgRP- induced hyperphagia are physiologically relevant for energetic need of the animals, whereas opioids are more involved in the hedonic or reward aspects of feeding and if so, MCH-induced hyperphagia may be for both satisfaction of energetic need and rewarding aspects of feeding. Our findings that MCH increased intake of sucrose and less potently glucose, but not saccharin, support the idea.

The MCH and MCH mRNA in the LH were reported up-regulated in genetically obese animals such as ob/ob mice, db/db mice and Zucker fatty rats. The findings that MCH and MCHR1 mRNA levels were highly correlated with sucrose intake indicate that endogenous levels of MCH may regulate sucrose intake of the animals.

In conclusion, our finding suggest that MCH-induced dipsogenic response is more related to caloric content than sweet taste per se. MCH may be a mediator for both energetic need and rewarding aspects of feeding and be involved in taste preference to sweet food and beverages, a well-known manifestation in diabetic animals and patients.





| 論文審査の結果の要旨 | | | |
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| 論文題目 | Melanin-Concentrating Hormone Enhances Sucrose Intake<br>メラニン凝集性ホルモンとショ糖摂取との関連 | | |
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（要旨は１，０００字～２，０００字程度）

メラニン凝集性ホルモン（MCH）は中枢神経系、特に摂食や摂水行動に関与する外側視床や Zona incerta に発現し、食物摂取やエネルギーバランスを調節している。MCH は食物摂取とは無関係に摂水を促進すると報告されており、また db/db マウスや OLETF ラットのような糖尿病モデル動物においては非糖尿病動物と比べて糖分摂取行動が亢進していることも報告されている。そこで本研究は MCH の甘味溶液摂取にかかわる特異な生理機能を明らかにすることを目的とした。

雄性 SD ラットにヒト MCH あるいはサケ MCH を脳室内投与すると摂食量および摂水量が増加したが、その作用はヒトよりもサケ MCH において強力であった。両 MCH による水分摂取の増加はアンギオテンシンⅡ（ANGⅡ）のアンタゴニストによる影響は認めなかった。ヒトおよびサケ MCH 脳室内投与ラットにおいては食事摂取とショ糖溶液摂水の増加が見られたが、アグーチ関連蛋白（AgRP）投与ラットにおいては食餌が存在するときには節食のみでショ糖溶液の摂水は認められなかった。

食餌のない条件下では MCH 投与ラットにおいてショ糖溶液やブドウ糖溶液の摂水の亢進が見られたが、人工甘味料でノンカロリーのサッカリン液の摂水は増加しなかった。RT-PCR にて視床下部の MCH と MCH 受容体 1（MCHR1）の mRNA の発現を 5 日間ショ糖溶液を摂水させたラットで検索したところ、摂取ショ糖量と MCH および MCHR1 の mRNA の発現との間に有意の正の相関関係が認められた。

今回の研究はラットにおいて、ヒトおよびサケ MCH が摂食や摂水促進作用を示すとともに、摂食とは無関係に摂水を促進させることを明らかにした。その効果はヒト MCH よりもサケ MCH で顕著であり、その原因として一部のアミノ酸配列の相違と、MCH 受容体との反応性の差異によるものと考えられた。ANGⅡは血圧や体液バランスの維持のための口渇や食塩摂取に呼応するホルモンであるが、今回の研究では MCH による摂水行動は ANGⅡアンタゴニストの影響は受けず、MCH は ANGⅡシステムとは無関係に独立して視床下部の神経系に影響を与えると考えられた。

次いで、甘味食品の摂食に大きな影響を与えると考えられている AgRP と MCH 系の比較を行った。各々を脳室内投与されたラットは食餌とショ糖溶液摂取において各々異なる行動を示し、両者は異なる別個の機序で摂食や摂水行動に関与している可能性が示唆された。また MCH 投与ラットにおいてはショ糖やブドウ糖溶液の摂水促進が見られたが、サッカリン液摂水の増加は認められず、MCH に誘導される過食は甘味摂取のみならずエネルギー確保にも関与していると考えられた。遺伝的に肥満である ob/ob マウスや db/db マウスにおいては視床下部における MCHmRNA 発現の亢進が報告されているが、本研究においてもショ糖摂取量に相関してその発現が亢進していたことより、内因性 MCH レベルはショ糖摂取量によって調節されている可能性も考えられた。

本研究は糖尿病患者やそのモデル動物にみられる食餌や甘味成分の過剰摂取の機序について、神経体液因子の作用を検討したものであるが、従来ほとんど行われなかったメラニン凝集性ホルモン（MCH）系の作用について重要な知見を得たものとして価値ある集積であると認める。よって、本研究者は博士（医学）の学位を得る資格があると認める。