The Impact study of motilin digestive function in hypothalamus paraventricular nucleus*

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ABSTRACT Objective: To investigate the effects of paraventricular hypothalamic nucleus （PVN）administration of motilin and motilin receptor antagonist GM-109 on ingestion and body weight gain in rats. Methods: Motilin and GM- 109 were injected in PVN at doses of 0.005-5 nmol. A significant increasing ingestion was observed 1h after PVN injection motilin 3nmol/rat and continued for 2h. Ingestion was measured by decreasing of pellets between the preweighted pellets and the uneaten pellets at 20 min, 1 and 2 h after drug. The mice were weighted .Daily PVN administration of motilin and GM-109, the weight of the rats were detected for 1week. Body weight, food intake were analyzed and compared with those of the rats of ACSF (NaCl, 138.9 mmol;KCl, 3.4 mmol; $CaCl_2$, 1.26 mmol; $NaHCO_3$, 4 mmol;$NaH_2PO_4 \cdot 2H_2O$, 0.6 mmol; and glucose, 5.6- mmol)-treated controls. Results: PVN administration of motilin 5nmol/rat increased significantly the food intake after 1 and 2 h. The body weight increased 7days later. Food intake increased significantly ($p<0.01$), however, body weight gained slight ($p>0.05$). However, which was not observed in PVN administration GM-109 at doses of 0.005-5nmol/rat, or ACSF -injected controls. These effects indicate that motilin is involved in the regulation of digestive motility and that motilin receptors are present in the brain and may have a role in the regulation of food intake. Conclusion: motilin modulates digestive movement, and promotes gastric emptying and appetitive effect. May be due to gastric emptying is frequent, no plenty of time to digest absorb, thus weight gain no significant differences.

Key word: Motilin; PVN; Food intake; GM-109; Brain

Chinese Library Classification (CLC): 282.71 Document code: A

Article ID: 1673-6273 (2011)03-460-05

Introduction

Feeding behavior is known to be regulated by many mediators and regulatory pathways in the brain and periphery. Now, there is growing evidence that food intake is regulated by neurotransmitters and neuromodulators including anorexigenic and orexigenic neuropeptides in the hypothalamic nuclei in interaction with blood-borne hormones and nutrients [1]. Motilin, a 22-amino-acid peptide released periodically from the mucosa of the upper small intestine during fasting, is known to stimulate gastrointestinal motility [2]. In endogenous motilin participates in the control of the interdigestive migrating motor complex (MMC) [3,4], Motilin may be one of the orexigenic peptides, at least when administered into the brain [5]. Ghrelin (previously known as the growth hormone secretagogue receptor; GHSR) is also released by the gut during fasting and may be regarded as motilin's cousin. ghrelin in certain experiments has been shown to have activity via a receptor that is distinct from the ghrelin receptor (e.g. increased feeding in rats after intracerebroventricular administration [6]. Its orexigenic and anabolic actions[7]. Ghrelin induces adiposity in rodents by increasing food intake and decreasing fat use [7]. Thus, peptide hormones play significant roles in energy homeostasis in the central nervous system [1]. Motilin immunoreactivity, motilin mRNA and the motilin receptor have been detected in the central nervous system-in the cortex, cerebellum, hippocampus, amygdala and hypothalamus [8-10], which suggests that motilin plays an integrative role as neurotransmitter and/or neuromodulator in the brain [11]. To investigate the effects motilin and motilin receptor antagonist GM-109 on ingestion and body weight gain in rats, we injected motilin and GM-109 into PVN to further specify the effects of possible of motilin.

1 Methods and methods

1.1 Animals

Twelve week old male Sprague-Dawley rats (Qingdao Marine Drug Institution) weighing180-250g were used in all experiments, which were housed in a temperature-controlled room (20℃) with lights on from 08.00 to 20.00 h. All animal were in a stress-free environment with constant temperature and humidity conditions. All rats were fed standard laboratory chow and tap wa-

*Foundation items :National Natural Science Foudation of China (No.30470642 and No.30670780); Shangdong Province Tackle Key Problems in Science and Technology Program(2008GG10002006); Qingdao Municipal Science and Technology Commision (05-1-JC-93) and Shangdong Province Health Department 266021 (2007-37)

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（Received: 2010-10-22 Accepted：2010-11-20）

ter available ad libitum. And the total number of rats was 60, which were randomly divided into 9 groups (n=6), experiments were carried out according to the guidelines for animal experimentation of Qingdao Medical School. All efforts were made to minimize animal suffering and to reduce the number of animal in experiments.

1.2 Material

Rabbit motilin and selective motilin receptor antagonist GM-109[12] (gift from Gut Hormone Laboratory, Leuven, Belgium) was microinjected into PVN.

1.3 methods

Before the experiments, they were anesthetized with Chloral Hydrate (3 ml/kg of a 10% solution, i.p.), hair around the skin area where the incision was planned was shaved, and skin was scrubbed with 70% alcohol. The animal was mounted on the center stereotaxic instrument (Narashige SN-3, Tokyo, Japan), and 24-gauge cannulas was inserted into PVN according to the brain stereotaxic atlas (1.8mm posterior to the bregma, 0.5 mm to the midline, 8 mm under the cranium surface) of SD rats. The entire exposed cannulas were covered with dental cement and dental base acrylic resin powder, which were served to affix the cannulas. A 27-gauge injection insert was attached to a microsyringe by PE-20 tubing. After the surgery, each rat received a daily peritoneal administration of benzylpenicillin sodium (2×10^4 units) [9] to resist infection.The rat recovered completely from the surgery after 3 d, would be used for the later experiment. After the experiment, the location of the cannula tip was confirmed by the injection of dye (Pontamine sky blue 0.5μl) and histological examination of frozen brain sections. More than 90% of the cannulas were properly placed. Experiments were started at 8:00 a.m. Motilin and GM-109 were dissolved in 4μl ACSF (NaCl, 138.9mmol; KCl, 3.4mmol; $CaCl_2$, 1.26mmol; $NaHCO_3$, 4mmol; $NaH_2PO_4 \cdot 2H_2O$, 0.6mmol; and glucose, 5.6mmol.) The injection doses was 0.005-5nmol daily. Similarly, daily doses of co-administration of motilin and GM-109 were 0.005-5nmol. Body weight of animals was measured at 8:00a.m everyday. Food intake was measured by placing the preweighed pellets in the cage and weighing the uneaten pellets at 20 min, 1 h and 2 h after drug administration for 1 week.

1.4 Statistical analysis

Results were presented as mean ± SD. Data were evaluated by ANOVA with repeated measures. Analysis of data was performed with t-test and one-way analysis of variance (SPSS17. O software package). P value which was less than 0.05 was considered to be statistically significant.

2 Results

Motilin increased food intake, 5nmol motilin, 4μ l significantly increased total food intake. After PVN injection of 5nmol/rat motilin 20min, the cumulative food intake compared with ACSF-treated controls of cumulative food intake ($p<0.05$,Fig. 1). After PVN injection of 5nmol/rat motilin 1h and 2 h , the cumulative food intake compared with ACSF-treated controls of cumulative food intake ($p<0.01$,Fig. 1). After PVN injection of 0.05nmol/rat motilin 2 h, the cumulative food intake compared with ACSF-treated controls of cumulative food intake ($p<0.05$, Fig. 1).PVN injection of motilin increased food intake. The highest dose of motilin, 5nmol, produced a significant increase in food intake at 1h and 2 h after PVN injection ($P < 0.01$). The lower dose of rat motilin, 0.05nmol, produced a delayed significant increase in food intake (Fig. 1). Therefore 5nmol /rat motilin appeared to be the threshold dose. Control PVN injection of rat ACSF had no effect (Fig. 1). After PVN co-injection of 5nmol/rat motilin and GM109 0.005nmol 20 min, the cumulative food intake compared with ACSF-treated controls of cumulative food intake ($p<0.05$, Fig. 2). After PVN co- injection of 5nmol/rat motilin and GM109 0.005nmol 1h and 2 h, the cumulative food intake compared with ACSF-treated controls of cumulative food intake ($p<0.01$,Fig.2). This effect of motilin (5nmol/rat) was antagonized by the simultaneous PVN administration of the motilin receptor antagonist GM-109 (0.005-5nmol/rat) in a dose-related manner ($p < 0.01$ at 1h and 2 h by doses of 5nmol motilin and 0.005nmol GM-109 or 5nmol motilin and 0.05nmol GM-109; Bonferroni's t-test; $p < 0.05$ at 2 h by doses of 5nmol motilin and 0. 5nmol GM-109 ; Bonferroni's t-test; Fig. 2). After 7days of treatment with motilin (0.05-5nmol/rat)and GM-109 (0.05-5nmol/rat), body weight increased [$F(8, 45)$, $p>0.05$, Bonferroni's t-test; Fig. 3,4.] compare with that of ACSF-treated controls (not significant). GM-109 is a cyclic peptide and a highly selective motilin receptor antagonist in the smooth muscle of the rabbit small intestine [13]. IP injection of motilin (0.005-5nmol /rat), in contrast did not increase food intake in nonfood-deprived rat. GM-109 alone at doses of 0.005-5nmol / rats had no effect on food intake[5].

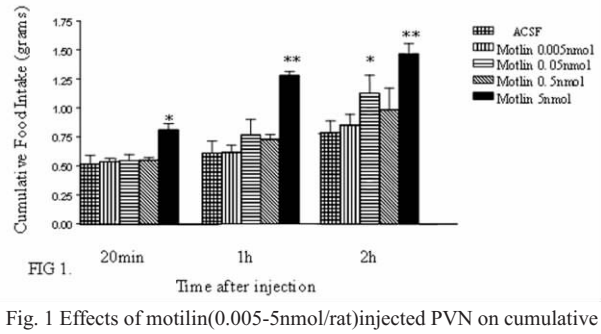

Fig. 1 Effects of motilin(0.005-5nmol/rat)injected PVN on cumulative food in nonfood-deprived rats. The number of rats used are 6 per each group. *$p<0.05$**$p<0.01$ compared to the ACSF control group by Bonferroni's t-test

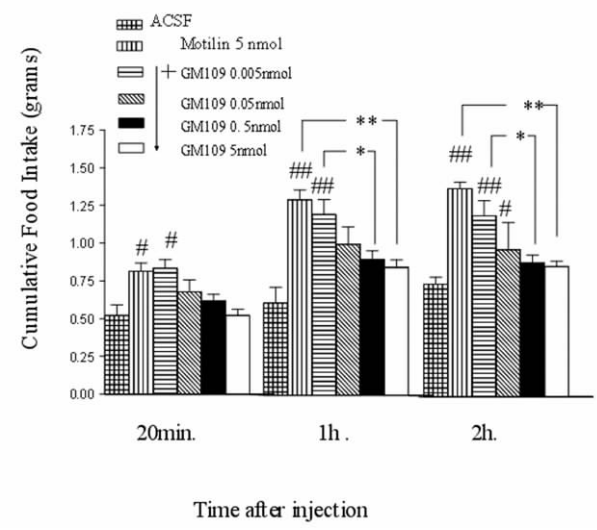

Fig. 2 Effects of motilin (5 nmol/rat) or motilin (5 nmol/rat) + GM109 (0.005-5nmol/rat) injected PVN on cumulative food intake in nonfood-deprived rats. The number of rats used are six per each group. # p＜0.05,##p＜0.01, the motilin (5 nmol/rat) alone group and the motilin (5 nmol/rat) +GM109 (0.005nmol/rat) compared to the ACSF control group by Bonferroni's t-test. * p ＜0.05, * *p ＜0.01, the motilin (5 nmol/rat) + GM109(0.5-5nmol/rat) compared to the motilin (5nmol/rat) + G M109(5nmol/rat) by Bonferroni's t-test.

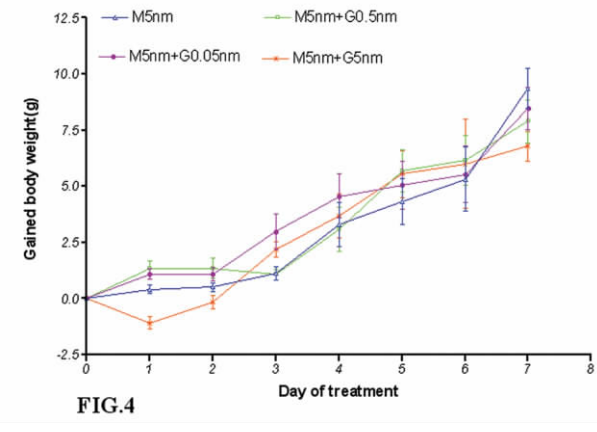

Fig. 4: Effects of motilin and a mixture of motilin and GM-109 microinjected into PVN on gained body weight. . Daily administration of the motilin 5nmol group compared to the mixture of motilin and GM-109 group by Bonferroni's t-test. p ＞0.05, no significant. Data are means± S. E. (n = 6)

3 Discussion

The present study demonstrated that PVN injections of motilin were highly effective in increasing food intake in non-food deprived rats. The aim of the present study was to investigate the effect of PVN motilin administration on food intake and weight gain in rats.

The hypothalamus is critical in the relaying of afferent signals from the gut and brainstem as well as processing efferent signals that modulate food intake and energy expenditure. The hypothalamus is subdivided into interconnecting nuclei, including Arc, paraventricular nucleus (PVN), ventromedial nucleus (VMN), dorsomedial nucleus (DMN) and lateral hypothalamic area (LHA). Neuronal pathways between these nuclei are organized into a complex network in which orexigenic and anorexigenic circuits influence food intake and energy expenditure.

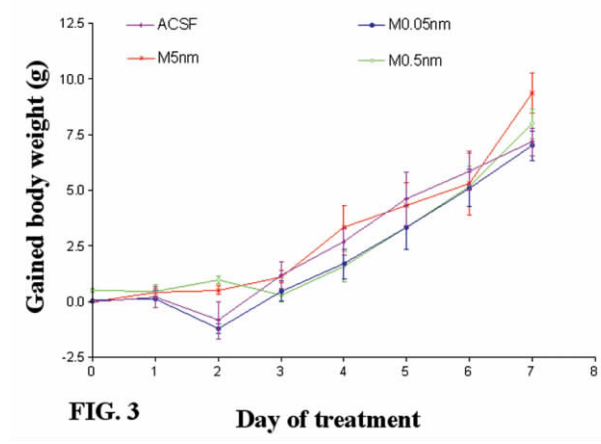

Fig. 3: Effects of motilin (0.05-5nmol/rat) injected PVN on gained body weight . Daily administration of the motilin(0.05-5nmol/rat)group compared to the ACSF control group by Bonferroni's t-test. p ＞0.05, no significant. Data are means± S.E. (n = 6).

The paraventricular nucleus (PVN) is adjacent to the superior part of the third ventricle in the anterior hypothalamus. The PVN is the main site of corticotrophin releasing hormone (CRH) and thyrotropin releasing hormone (TRH) secretion. Numerous neuronal pathways implicated in energy balance converge in PVN, including major projections from NPY neurons of the ARC, Orexins, POMC derivative a-melanocyte stimulating hormone (a-MSH) and the appetite stimulating peptide galanin. Thus PVN plays a role in the integration of nutritional signals[14]. PVN motilin administration increased feeding in a dose-dependent manner. Motilin has previously been found to have induced appetite effect in rats [5]. Previous work showed that motilin increased food intake when injected into intracerebroventricular (ICV) [15]. Motilin might modulate neuronal excitability of the myenteric plexus, leading to the control of interdigestive migrating contractions. Feeding behavior is regulated by neurotransmitters and neuromodulators in the central nervous system as well as blood-borne factors. Ghrelin, like motilin, was capable of inducing phase 3 of the MMC and of accelerating gastric emptying in man and in rodents [16-20]. In rats, the motor effects in vivo are blocked by vagotomy [17], It has become evident that motilin exerts its effect in the period after digestion and absorption have finished, and induces strong phasic contractions in the stomach, and stimulates enzyme secretion from the stomach and pancreas, and also stimulates the endocrine pancreas to release somatostatin [21,22,] IP injection of motilin (0.005-5 nmol/rat) did not increase food intake in nonfood-deprived mice. GM-109 alone with the doses of 0.005-5nmol/mouse had no effect on food intake [5]. The hypothalamic paraventricular nucleus and

PVN motilin participate in the central control of gastric motility[23]. Thus, based on previous and present data, it can be concluded that PVN injection motilin stimulates contractions, induces Phase II-I-like contractions migrate, motilin may be one of the orexigenic peptides, at least when administered into the brain [5]. Therefore, motilin might attributable to gastric emptying, thus stimulate food intake , on the other hand, because of gastric emptying maybe fast and frequent , decreasing the volume of gastric contents, food without enough time to absorb , therefore, body weight gain not significant[24].

Acknowledgements

The authors appreciate the assistance of Dr. T.L. Peeters Gut Hormone Laboratory, Leuven, Belgium for supplying GM109.

References

[1] Masuda Y, Tanaka Y, Inomata N, et al. Ghrelin stimulates gastric acid secretion and motility in rats [J]. Biochem Biophys Res Commun, 2000, 276:905-908

[2] Boivin M, Raymond M, Riberdy M. Plasma motilin variation during the interdigestive and digestive states in man [J]. Gastr Mot, 1990, 2: 240-246

[3] Itoh Z. Motilin and clinical application [J].Peptides, 1997, 18: 593-608

[4] Vantrappen G, Janssens J, Peeters TL, et al. Motilin and the interdigestive migrating motor complex in man [J]. Dig Dis Sci, 1979,24: 497-500

[5] Schick R, Schusdziarra V. Modulation of motilin-induced somatostatin release in dogs by naloxone. [J]. Peptides, 1985, 6:861-864

[6] Toshinai, K. et al. Des-acyl ghrelin induces food intake by a mechanism independent of the growth hormone secretagogue receptor [J]. Endocrinology, 2006, 147:2306-2314

[7] Tscho¨ p M, Smiley DL, Heiman ML. Ghrelin induces adiposity in rodents. [J]. Nature, 2000, 407:908-913

[8] Guan YF, Tang M, Jiang ZY, et al. Excitatory effects of motilin in the hippocampus on gastric motility in rats [J]. Brain Research,2003,984: 33-41

[9] Depoortere I, De Clercq P, Svoboda M, et al. Identification of motilin mRNA in the brain of man and rabbit. [J]. Peptides,1997, 18: 1497-1503

[10] Depoortere I, Van Assche G, Peeters TL. Distribution and subcellular localization of motilin binding sites in the rabbit brain [J].Brain Res, 1997,777: 103-109

[11] Hameed S, Dhillo WS, Bloom SR. Gut hormones and appetite control [J].Oral Dis, 2009, 15: 18-26

[12] Takanashi H, Yogo K, Ozaki K, et al. GM-109: a novel, selective motilin receptor antagonist in the smooth muscle of the rabbit small intestine [J]. Pharmacol. Exp. Ther, 1995, 273: 624-628

[13] Yan CL, Wang SB, Jiang ZY. Effects of motilin in the paraventricular hopothalamic nucleus on gastric motility [J]. applied physiology of China journal, 2002,18:317-320

[14] Goro K, Akihiro Aa, Akio I. Roles of pancreatic polypeptide in regulation of food intake[J]. Peptides, 2002,23: 323-329

[15] Asakawa A, Inui K, Momose N, et al. Motilin increases food intake in mice. [J] Peptids, 1998, 19: 987-990

[16] Xu L, Depoortere I, Tomasetto C, et al. Evidence for the presence of motilin, ghrelin, and the motilin and ghrelin receptor in neurons of the myenteric plexus. [J].Regulatory Peptides, 2005, 124: 119- 125

[17] Fujino K, Inui A, Asakawa A, et al. Ghrelin induces fasted motor activity of the gastrointestinal tract in conscious fed rats [J]. Physiol, 2003, 550:227- 240

[18] Tack J, Depoortere I, Bisschops R, et al. Influence of ghrelin on gastric emptying and meal-related symptoms in gastroparesis [J]. Gastroenterology, 2004, 126: 479-485

[19] Trudel L, Tomasetto C, Rio MC, et al. Ghrelin/motilin-related peptide is a potent prokinetic to reverse gastric postoperative ileus in rat [J]. Gastrointest Liver Physiol, 2002, 282:948-952

[20] Depoortere I, De Winter BY, Thijs T, et al. Comparison of the prokinetic effects of ghrelin, GHRP-6 and motilin in rats in vivo and in vitro [J]. Gastroenterology, 2003, 124: 576-580

[21] Aizawa I, Itoh Z, Harris V, et al. Plasma somatostatin-like immunoreactivity during the interdigestive period in the dog [J]. Clin.Invest, 1981,68:206-213

[22] Chung S. Greenberg G. Diamant N. Vagal control of fasting somatostatin levels. [J].Neurogastroenterol, 1995, 7:73-78

[23] Yan CL, Wang SB. Zhengyao Jiang, et al. Gastric motility effects study of motilin in the paraventricular hypothalamic nucleus [J]. Appl Physiol ,2002,18: 317-320

[24] Chung S. Greenberg G. Diamant N. Vagal control of fasting somatostatin levels. [J].Neurogastroenterol, 1995, 7:73-78

下丘脑室旁核胃动素对消化功能影响的研究 *

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摘要 **目的：**研究下丘脑室旁核注入胃动素及其拮抗剂对大鼠消化功能和体重增长的研究。**方法：**将剂量为 0.005-5nmol 的 motilin 和 GM109 注入大鼠下丘脑室旁核，1 小时后可观察到大鼠摄食量显著增加并持续到两小时后。进食量的计算是通过预先称量好的鼠粮和应用药物 20 分钟、1 小时、两小时后剩余数量比较而得出。实验持续一周。将实验组和对照组的进食量和体重进行比较。**结果：**室旁核注入胃动素 5nmol 的实验组和合并应用 GM1090.005nmol 的实验组在应用药物后 1 小时和 2 小时，可观察到摄食量显著增加($p<0.01$)，一周后体重也增加($p>0.05$)，然而摄食量的增加有显著性差异，体重的增加并无显著性差异。其他实验组也没有观察到显著性差异。**结论：**胃动素有调节消化运动，促进胃肠排空，促进食欲的作用。可能由于胃肠排空是频繁的，没有充裕的时间消化吸收，从而体重增加无显著性差异。

关键词：胃动素；室旁核；摄食；GM109；脑

中图分类号：282.71 **文献标识码：**A **文章编号：**1673-6273(2011)03-460-05

* 基金项目：国家自然科学基金项目资助(30470642)；山东省科学与技术项目(2008GG10002006)；
青岛市科技基金(05-1-JC-93)与山东省卫生局科技基金 (266021(2007-37)

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(收稿日期：2010-10-22 接受日期：2010-11-20)