Effection of GLP-1 Injection in Paraventricular Nucleus on the Expression of GLP-1 Receptor and Gastric Emptying in Diabetic Rats*

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ABSTRACT Objective: To investigate the effection of GLP-1 injection GLP-1 in paraventricular nucleus on the expression of GLP-1 receptor and gastric emptying in diabetic rats. Methods: Thirty Wistar rats were randomly divided into three groups: Group 1: normal control group (NC group), Group 2: diabetic mellitus group (DM group) and Group 3: GLP-1 treated group (GLP-1 group). All of rats were posited a cannula in PVN, the rats of Group 2 and Group 3 were induced by intraperitoneal injection of streptozotocin (STZ). The rats of Group 3 were injected 0.5μg/0.5μl GLP-1 and isovolumic normal saline for Group 1 and Group 2 in PVN after 7-day recovery period following surgery. Gastric emptying was measured by intragastric administration of methyl cellulose-phenol red solution. The GLP-1 levels in the hypothalamus were measured by EISIA method, the expression levels of GLP-1R mRNA in the hypothalamus were measured by semi-quantitative RT-PCR. Results: The gastric emptying of Group 2 showed significantly accelerated compared with that Group 1 ($P<0.05$). which decreased significantly in Group 3 compared to Group 2 ($P< 0.05$), There was no statistical difference between Group 2 and Group 1 ($P>0.05$). The expression of GLP-1 and GLP-1R mRNA in hypothalamus of Group 3 was increased significantly compared with in the other two groups and no difference between Group 2 and Group 1 ($P>0.05$), which showed a negative correlation with decrease of gastric emptying ($P<0.05$). Conclusion: GLP-1 Injection in PVN delays acceleration of gastric emptying during the early stage of diabetic rats. The expression of GLP-1 receptor in the hypothalamus is promoted by GLP-1 may be the mechanism.

Key words: Diabetes mellitus; Paraventricular nucleus; Gastric emptying; GLP-1; Receptor

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Glucagon-like peptide 1 (GLP-1) is an intestinal hormone that is secreted by mucosal L cells located predominantly in the distal intestine , and is also expressed in pancreatic alpha cell, as well as in hypothalamus and brainstem [1] , GLP-1R has been identified and is expressed in a wide range of tissues including pancreas, lung, heart, kidney, stomach, intestine, pituitary, skin, nodose ganglion neurons of the vagus nerve, and several regions of the central nervous system (CNS) including the hypothalamus and nucleus of the tractus solitarius, reticular nucleus [2]. The experimental data indicate that the inhibitory effect of GLP-1 on gastric emptying is possiblely mediated by binding its receptor located in the CNS and/or peripheral nervous system (PNS) [3].

It is now thought that GLP-1 mediated primarily gastric emptying by peripheral vagus nerve. However, it remains unclear how central GLP-1 delays gastric emptying in diabetes mellitus. Increasing evidence suggests that the hypothalamic paraventricular nucleus (PVN) participates in the gastric emptying action of GLP-1 [4]. The PVN contains a high density of GLP-1-immunoreactive nerve fibers and terminals GLP-1 receptor mRNA. It has been shown that GLP-1 receptors are existed on 80% of CRF immunoreactive nerve fibers in the PVN [5]. This led us to hypothesize that the action of GLP-1 on gastric emptying is associated with PVN in the brain. This study investigated whether central GLP-1 affects gastric emptying and examined the expression of GLP-1 receptors in rats. The aim of our study was to study the central mechanism of Central administration of GLP-1 on gastric emptying in PVN of hypothalamus.

1 MATERIAL AND METHODS

1.1 Animals and groups

Male Wister rats, weighing 250-300 g, were housed individually in standard plastic rodent cages and allowed a 1-wk adaptation period before the experiments. The animal holding room was maintained at (22±1.0) ℃. The rats were given free access to laboratory chow and tap water ad libitum. Thirty Wistar rats were randomly divided into three groups: normal control group (NC group), diabetic mellitus group (DM group), GLP-1 treated group (GLP-1 group).

1.2 Induction of diabetes

Rats were fasted for 16 hours and received intraperitoneal injection of streptozotocin (STZ) at 60mg/kg body weight in citrate buffer (PH 4.4), Control rats received intraperitoneal injection of citrate buffer alone. All animals were given access to water and suitable rat chow ad libitum. Diabetic rats showed a nonfasting gluco-

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se concentration of≥16.7mmo/L in tail vein blood on the 3th days after STZ injection.

1.3 Surgical procedure

Before surgery, each rat was injected intraperitoneally with 80 g/L chloralic hydras (400mg/kg). With the upper incisor bar of the stereotaxic instrument positioned at 3.0mm below the interaural line, the tip of a stainless steel 15-gauge guide cannula was positioned PVN, and the coordinates of PVN for the placement of cannula were taken from the atlas of Paxinos and Watson. as follows: 1.6mm caudal to bregma, 0.2mm lateral to the midline, and 7.2mm below the surface of the skull. The shaft of each unilateral guide cannula was affixed to the skull with stainless steel screws and a pedestal of dental acrylic. After surgery, each rat was injected intramuscularly with penicillin (80,000 units each). A 7-day recovery period followed surgery, during which the rats were weighed daily and had continuous access to water and food pellets in the home cage.

1.4 Measurement of gastric emptying [6]

Two weeks after STZ injection, rats were fasted for 16 hours but allowed water ad libitum up to 2 hours before the experiments. The emptying of a liquid solution (1.5% methyl cellulose) was measured using phenol red (50mg/l00ml). Fifteen minutes after oral administration of 2ml of the liquid solution, rats were intraperitoneal anesthetized with 80g/L chloral hydrate (400mg/kg). Immediately after the stomach was exposed by laparotomy, the stomach was then ligated at the pylorus and cardiac and opened along the greater curvature of the stomach. The stomach contents was washed with distilled water and fixed volume of 20m1. Then 20ml of 0.5 mol/L NaOH was added mixing and blending. After standing for l h, 5ml of supernatant was added with 0.5ml of 20% trichloroacetic acid. The mixture was centrifuged at 3500rpm for 10min and the supernatant were measured with the spectrophotometer at 560nm wave length absorbance value. An alternative phenol red-methyl cellulose solution 2ml was joined with 18ml of distilled water, 20ml of 0.5mol/L NaOH, 4ml of 20% trichloroacetic acid mixing and the measured absorbance value was the standard phenol red absorbance. Rat gastric emptying rat = (1- measured phenol red absorbance /standard phenol red absorbance) ×100%.

1.5 Measurement GLP−1 in the hypothalamus

Each rat was injected intraperitoneally with 80g/L chloralic hydras (400mg/kg), The brain was exposed with cut along the midline incision. The left half of hypothalamus was stored at -70℃ for RT-PCR. The right half of hypothalamus was boiled in saline for 5min and dried with filter paper. It was homogenized plus 0.5mL 1mol/L HCl and standing at room temperature for 100min , then added 0.5mL 1 mol/L NaOH and centrifuged at 4℃ (4000rpm) for 15min and supernatant was stored at -20 ℃ for ELISA. The GLP-1 levels were measured by enzyme-linked immunoassay method.

1.6 Determination of GLP−1R mRNA in the hypothalamus with Semi−quantitative RT−PCR

Total cellular RNA was prepared from hypothalamus using Trizol according to the manufacturer's protocol. Singlestrand cDNA was synthesised from the individual samples of total RNA at 0.5μg using PrimeScript RT-PCR Kit. After addition of each set of primers (final concentration 0.4μmol/l) and template DNA to the master mix, RT-PCR was performed with PrimeScript RT-PCR Kit (TaKaRa company).The PCR protocol was as follows: predegeneration for 5min at 95℃, denaturation for 30s at 95℃, annealing for 30s at 56℃, extension for 30s at 72℃, followed by 35 cycles, terminal extension at 72℃ after 10min and stored at 4℃. The specific oligonucleotide primer sequences are shown as follow. GLP-1R forward primer: 5'-AGT AGT GTG CTC CAA GGG CAT-3', reverse primer: 5'-AAG AAA GTG CGT ACC CCA CCG-3'; β-actin forward primer: 5'-GCC CCT CTG AAC CCT AAG-3', reverse primer: 5'-CAT CAC AAT GCC AGT GGT A-3'. To visualise gene expression, individual DNA fragments were electrophoresed on a 1.5% agarose gel and treated with ethidium bromide. The signal for GLP-1R mRNA was normalized to the signal of the housekeeping gene β-actin and the results were expressed as the GLP-1R mRNA/β-actin ratio.

1.7 Statistical analysis

Data were expressed as mean± SE. To analyze the data statistically, an independent sample t-test was used to determine which means were significantly different from the mean of the control groups. A correlation analysis was used to confirm the relation between GLP-1R mRNA expressions and gastric emptying. A11 calculations were performed with SPSS 17.0 for Windows. P- values less than 0.05 were considered to indicate statistical significance.

2 RESULTS

2.1 Gastric emptying

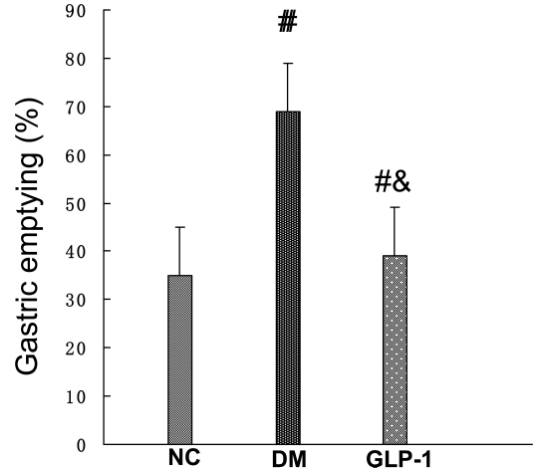

Fig.1 Comparison of the gastric emptying in four groups
#P < 0.01 VS NC group，&P < 0.01 VS DM group

Two weeks after the STZ injection，the liquid gastric emptying accelerated significantly in DM group compared to those in NC

group (P< 0.05). The liquid gastric emptying slowed significantly in GLP-1 group compared to those in DM group (P< 0.05), and were no statistical difference in GLP-1 group compared to those in NC group (P>0.05) (Fig.1).

2.2 Levels of GLP−1 in the hypothalamus

Two weeks after the STZ injection, the GLP-1 levels in the hypothalamus increased significantly in GLP-1 group compared to those in NC and DM group(P<0.05); The GLP-1 levels in the hypothalamus were no statistical difference in DM group compared to those in NC group (P>0.05)

2.3 The expression of GLP−1R mRNA in hypothalamus

The expression of GLP-1R mRNA in hypothalamus of GLP-1 group was increased significantly compared with that of the DM group and NC group, which showed a negative correlation with decrease of gastric emptying (P< 0.05), There was no statistical difference between DM group and NC group (P> 0.05) (Table1 and Fig.2).

Table 1 The GLP-1 levels in the hypothalamus and GLP-1R/β-actin in the hypothalamus in three groups ($\bar{x}\pm s$)

Group	N	Weight (g)	Blood-glucose	GLP-1(ng/g)	GLP-1R/β-actin
NC	10	341±29.8	4.9±1.2	32±4	0.75±0.11
DM	8	274±48.3[a]	27.3±2.6[a]	30±7[a]	0.81±0.17
GLP-1	7	254±30.0	25.5±3.7[a]	45±10[b]	1.23±0.34[b]

Note: [a]P< 0.05 vs NC ; [b]P< 0.05 vs DM

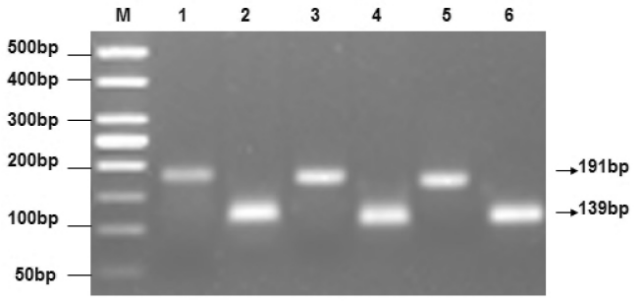

Fig.2 The expression of GLP-1R mRNA in the hypothalamus in three groups From left to right: Marker，NC，NC-β-actin，DM，DM-β-actin，GLP-1, GLP-1-β-actin

3 DISCUSSIONS

GLP-1 is a posttranslational product of the proglucagon molecule that is secreted by distal intestinal L cells in response to food entering the gut, particularly carbohydrate-rich foods. GLP-1 exerts a broad range of actions, including stimulation of insulin biosynthesis inhibition of glucagon secretion, inhibition of gastric emptying and acid secretion, reduction of food intake and trophic effects on the pancreas [7-8]. In fact, it has been suggested that the actions of GLP-1 to slow gastric emptying, and thereby the entry of nutrients into the small intestine to delay their absorption, may outweigh its insulinotropic and glucagonostatic effects [9]. Collectively, the experimental data indicate that the inhibitory effect of GLP-1 on gastric emptying and acid secretion is mediated by the vagus nerve and involves GLP-1Rs located in the CNS and/or PNS.

The mechanism of regulating gastric emptying is thought to be primarily a function of the pressure gradient between the stomach and the duodenum. In pharmacological dosages, GLP-1 relaxes the proximal stomach, inhibits antral and stimulates pyloric motility [10]. The resulting retardation of gastric emptying is an important mechanism by which GLP-1 minimizes postprandial glycaemic excursions [11]. Animal data suggest that GLP-1 may exert its action on gastric emptying by inhibiting vagal nerve activity [12]. The vagus regulates gastroduodenal motility via cholinergic excitatory and non-adrenergic non-cholinergic inhibitory inputs, the latter is mediated by nitric oxide. Inhibition of nitric oxide synthase blunted the effect of exogenous GLP-1 on gastric accommodation after an oral meal in humans, suggesting that nitric oxide partly mediates the effect of exogenous GLP-1[13].

Increasing evidence suggests that the hypothalamic paraventricular nucleus (PVN) participates in the activities of gastric emptying inhibition of exogenous GLP-1 [14]. The PVN contains a high density of GLP-1-immunoreactive nerve fibers and terminals [4], exhibits GLP-1 binding, and contains GLP-1 receptor mRNA. Moreover, central infusion of GLP-1 results in the expression of c-fos within the PVN [15]. It has been shown that central administration of GLP-1 delays liquid gastric emptying via non-adrenergic, non-cholinergic neurons in rats [16].

Our study shows that gastric emptying is decreased significantly of GLP-1 group compared with that of DM group, The GLP-1 levels and the GLP-1R mRNA expression levels in the hypothalamus increased significantly compared with those DM group, which showed a negative correlation with decrease of gastric emptying. These observations suggest that GLP-1 inhibits gastric emptying in the diabetic rats. Because of its ease of penetration through the blood-brain barrier, central acting GLP-1 acts via GLP-1 receptor to delay gastric emptying probably. It has also been reported that gastric emptying is accelerated in the early stages of STZ-induced diabetic rats. Gastrointestinal hormone and electrical activity of stomach are likely to play a major role. Our study coincides with this idea.

In summary, GLP-1 has a significant role in the inhibition of gastric emptying in diabetes. GLP-1 combine with receptor is one of its possible mechanisms. It is not clear that the peripheral GLP-1 receptor expression levels and the relationship with gastric emptying. The further study in GLP-1 helps to understand the value of the treatment of diabetes, and is especially significant in the prevention and treatment of gastrointestinal complications and with other aspects of the blood glucose control.

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下丘脑室旁核注射 GLP-1 对糖尿病大鼠中枢 GLP-1 受体表达与胃排空的影响 *

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摘要 **目的**:探讨下丘脑室旁核(hypothalamic paraventricular nucleus,PVN)注射 GLP-1 (胰高血糖素样肽 -1) 对糖尿病大鼠胃排空的影响及机制。**方法**:30 只 Wistar 大鼠随机分为正常对照组 (NC 组)、糖尿病组(DM 组)和 GLP-1 干预组 (GLP-1 组),每组各 10 只。DM 组和 GLP-1 组腹腔注射链脲佐菌素,三组大鼠均 PVN 区埋置套管,恢复 7d, GLP-1 组微量注射 0.5μg/0.5μl 的 GLP-1,NC 组和 DM 组大鼠 PVN 区微量注射等体积生理盐水。甲基纤维素 - 酚红灌胃法检测胃排空;半定量 RT-PCR 检测大鼠下丘脑 GLP-1RmRNA 的表达。**结果**:DM 组胃排空率较 NC 组明显升高 (P<0.05), GLP-1 组胃排空明显低于 DM 组 (P<0.05), GLP-1 组和 NC 组差异无统计学意义 (P>0.05)。 GLP-1 组下丘脑 GLP-1RmRNA 的表达明显高于 DM 组和 NC 组 (P<0.05),并与胃排空率成负相关 (P<0.05)。DM 组和 NC 组差异无统计学意义 (P>0.05)。**结论**:PVN 区注射 GLP-1 可以抑制糖尿病大鼠早期胃排空加速,作用机制可能和促进下丘脑 GLP-1 受体表达有关。

关键词 糖尿病;室旁核;胃排空;胰高血糖素样肽 -1;受体

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