대한의생명과학회지 : 제6권 제4호 2000
Korean J. Biomed. Lab. Sci
Vol. 6, No. 4, p.229~235, December, 2000

Effects of Cephalic Glucopenia on Insulin and Glucagon Secretion in Central Nervous System-Intact Pancreas Perfused Rats

Hyun Ju Choi†

Department of Medical Laboratory Science, School of Biomedical Science and Engineering, Inje University, Kimhae 621-749, Korea

Abstract: *In situ* brain-pancreas perfusion was performed on male adult Sprague-Dawley rats, of which the central nervous systems (CNS) were intact during the perfusion procedure. The modified Krebs-Ringer buffer with 100 mg/dL of glucose and 20 mM of arginine was perfused for 30 min. In the experimental groups, a cephalic glucopenia was induced at 0 min (GLP1 group) or at 16 min (GLP2 group). The glucopenia was not induced in the control (CONT group). Insulin and glucagon concentrations in the effluent samples from the pancreas were measured using a RIA method. In all three groups, the first and second phases in the dynamics of the insulin and glucagon secretion were observed, which was a typical biphasic secretory pattern. The amount of insulin secretion tended to decrease in the GLP1 and GLP2 groups, but there was no statistically significant difference among the groups. However, the amount of glucagon secretion during 0~15 min of the perfusion period in the GLP1 group was greater as compared to the CONT group ($p<0.05$). The amount of glucagon secretion during 16~30 min of the perfusion period in the GLP2 group tended to be greater as compared to the CONT group, however there was no statistical significance. These data indicate that the cephalic glucopenia stimulates the direct secretion of glucagon from the pancreas during the early period of perfusion in the CNS-intact pancreatic perfused rats.

Key Words: Cephalic glucopenia, Pancreatic hormone, *in situ*-brain pancreas perfusion

INTRODUCTION

The stimulation-secretion coupling in the pancreatic β-cells is affected by various metabolites. The primary metabolic secretagogoues for the insulin secretion is a glucose, a crucial source for the energy supply in the brain. Therefore, the cephalic glucopenia may modulate the hormonal secretion from the pancreas[6]. It has been reported that glucagon secretory response to the glucose deprivation does not appear to be critically dependent upon the central nervous system (CNS)[20,21]. However, the CNS is involved in the regulation of insulin and glucagon secretion from the pancreas, as evident by the influence of the ventromedial and ventrolateral hypothalamic nuclei[17]. Indeed, it has been reported that the glucagon secretion is modulated by the sympathetic nervous system1[2,3,13,15,19] and parasympathetic nervous system[1,4]. Besides, the pancreas islet cells are highly innervated through the autonomic nervous system, and the nerve fibers terminate in the pericapillary space within the capillary basement membrane, or the nerve fibers are closely apposed to endocrine cells

*Received: October 6, 2000
Accepted after revision: December 4, 2000
†Corresponding author: e-mail: chj@ijnc.inje.ac.kr

in the pancreas[5]. Therefore, the neural inputs from the brain to the pancreas could be important in the regulation of insulin and glucagon secretion from the pancreas. A demonstration of the cephalic glucopenic influence on the insulin and glucagon secretion via direct neural innervation of the pancreas in the CNS-intact rats has yet to be reported. Therefore, this study was to investigate effects of the cephalic glucopenia on a direct insulin and glucagon secretion using an *in situ* brain-pancreas perfusion.

MATERIALS AND METHODS

Animals

Adult male Sprague-Dawley rats (300~350 g body wt) were fed the rodent pellet diet and water *ad libitum* and maintained in a 12 hour light-12 hour dark cycle at a temperature of 23~25℃. Animals were in the fed state. For the animal anesthesia, chloral hydrate (350 mg/kg body wt, Sigma, USA.) was injected intraperitoneally. Chloral hydrate, $CCl_3CH(OH)_2$ is a sedative hypnotic drug which is used for deep sedation. Chloral hydrate is slow in crossing the blood-brain barrier[12], and there is little or no evidence of preliminary excitation prior to the onset of depression. In therapeutic doses, it has little effect on respiration and blood pressure[11]. Animals were divided into three groups: control group (CONT, n=12) in which glucopenia was not induced, GLP1 group (n=6) in which a cephalic glucopenia was induced during the early period of perfusion (0 min), GLP2 group (n=6) in which a cephalic glucopenia was induced during the later period of perfusion (16 min).

Perfusate preparation

The components of the perfusate were as follows: 0.18% albumin, 4.00% of dextran, 100 mg/dL glucose, 0.11 M of $CaCl_2$, 0.154 M of KCl, KH_2PO_4, $NaHCO_3$, NaCl, and $MgSO_4$. Arginine (20 mM), a stimulator of glucagon secretion, was added using an infusion pump during the 0~30 min of the total perfusion period. The perfusate was continually oxygenated with a 95% O_2-5% CO_2, and maintained 37℃ using an external heating source. Perfusate inflow rate was maintained at 5 ml/min.

In situ Brain-Pancreas Perfusion Procedure

In situ brain-pancreas perfusion method was used since it allows the brain, heart, and lung to remain functionally intact, thus retaining the ability of the brain to modulate endocrine secretion via the direct CNS innervation to the pancreas. In the perfusion preparation, a midline laparotomy extending from the symphysis pubica to the xiphoid process was performed. The pancreatic vasculature was separated by ligating the aorta proximal and distal to the origin of the celiac artery, prehepatic vena cava, posthepatic vena cava, portal vein, and hepatic artery. The perfusate inflow began via the heparinized celiac arterial cannula. Total outflow was collected via a heparinized portal vein cannula, and its volume was measured. Viability of the CNS component of the preparation was determined by monitoring the vasoconstrictional changes. The total venous effluent was collected for 30 min with 1~5 min time intervals. The effluent samples were placed in tubes containing aprotinine (protease inhibitor), and all samples were kept on ice until centrifuged (10 min at 4℃, 3000 rpm). The supernatant was decanted and frozen at -20℃ until assayed.

Insulin and glucagon radioimmunoassay

Insulin levels in the perfusion effluents samples were determined by using a Desbuquois and Aurbach technique[9]. Purified rat insulin (Novo, Copenhagen, Denmark) was used as the reference standard and human ^{125}I insulin (Amersham Co. USA) was used as a tracer. Glucagon levels in the effluents samples were determined by a method of

Table 1. Amount of insulin secretion (μg/300 g body wt) during the perfusion period

Group \ Time	0~15 min	16~30 min
CONT[a]	9.70±1.55	7.76±1.71
GLP1[b]	6.54±0.71	5.94±1.10
GLP2[c]	8.10±1.71	7.05±0.81

Values are means±SE.
Values in the same row are not significantly different at $p<0.05$.
[a] CONT: a control group without glucopenia
[b] GLP1: a group with glucopenia at 0 min
[c] GLP2: a group with glucopenia at 16 min

Table 2. Amount of glucagon secretion (ng/300 g body wt) during the perfusion period

Group \ Time	0~15 min	16~30 min
CONT[a]	35.47±4.18	28.13±3.83
GLP1[b]	67.34±15.39†	35.82±7.67
GLP2[c]	37.91±3.63	39.86±4.48

Values are means±SE.
†: value of 0~15 min in the GLP1 is significantly different from those in the CONT and GLP2 ($p<0.05$).
[a] CONT: a control group without glucopenia
[b] GLP1: a group with glucopenia at 0 min
[c] GLP2: a group with glucopenia at 16 min

Unger et al.[23] using antibody 04A antiserum (Southwestern Medical School, University of Texax, USA.). Radioisotope detection was carried out using a Cobra auto gamma counting system (Hewlett Packard Instrument Co, USA). The cpms were counted from triplicated standard tubes and duplicated sample tubes, and count of the non specific bounding was subtracted. The bound/free triplicates for each point on the standard curve were averaged and plotted vs insulin or glucagon amount. For each sample duplicate, the bound/free was read off as insulin or glucagon amount (ng/ml). Insulin and glucagon secretion rates (ng/min) were calculated by using the measured perfusate effluent flow rate (ml/min) and the assayed insulin and glucagon concentrations (ng/ml).

Statistical analysis

All data were expressed as means±SE. The ANOVA and Scheffe F-test were used to determine a statistical significance between mean values in each group at $p<0.05$.

RESULTS

Amount of insulin secretion during the 30 min of perfusion period was shown in Table 1. During the first 15 min of perfusion period, the amount of insulin secretion in the CONT and GLP1 groups were 9.70±1.55 μg/300 g body wt, 6.54±0.71 μg/300 g body wt, respectively, and there was no statistical significance between the two groups. During the next 16~30 min of perfusion period, amount of the insulin secretion in the GLP2 group was 7.05±0.81 μg/300 g body wt and there was no statistical significance as compared to the other two groups (7.76±1.71 μg/300 g body wt in the CONT, 5.94±1.10 μg/300 g body wt in the GLP1). These data show that the cephalic glucopenia does not affect the insulin secretion from the pancreas in the CNS-intact rats significantly.

Amount of glucagon was shown in Table 2. During the first 0~15 min of period, the amount of glucagon secretion in the GLP1 group was the greatest as 67.34±15.39 ng/300 g body wt and was significantly greater than those in the CONT group (35.47±4.18 ng/300 g body wt) ($p<0.05$). In the GLP2 group, the 0~15 min was a period before induction of the glucopenia, and the amount of glucagon secretion during this period was 37.91 ±3.63 ng/300 g body wt. This amount was similar, as expected, to the amount during the 0~15 min in the CONT group (35.47±4.18 ng/300 g body wt). During next 16~30 min of period, the amount of glucagon secretion in the GLP2 group seems to be the greatest as 39.86±4.48 ng/300 g body wt,

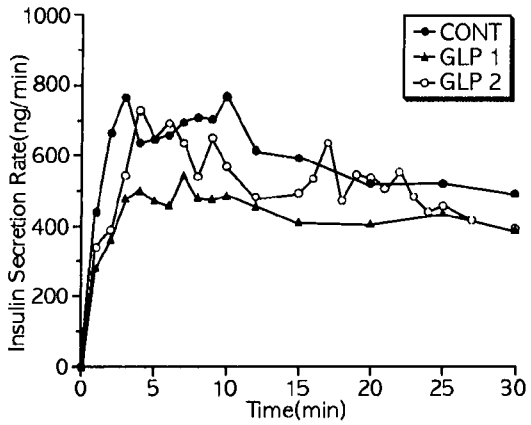

Fig. 1. Dynamics of the insulin secretion from pancreata of the CNS-intact rats. CONT: a control group without glucopenia, GLP1: a group with glucopenia at 0 min, GLP2: a group with glucopenia at 16 min.

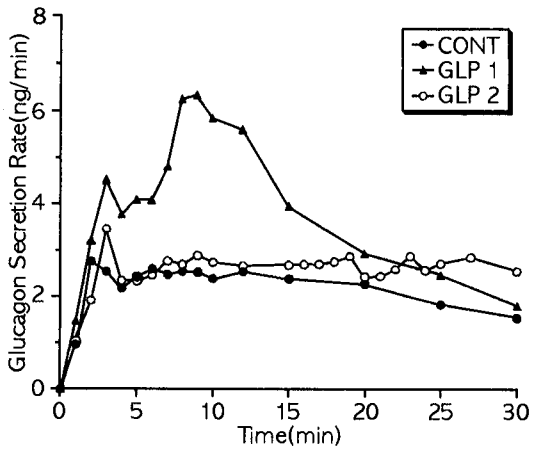

Fig. 2. Dynamics of the glucagon secretion from pancreata of the CNS-intact rats. CONT: a control group without glucopenia, GLP1: a group with glucopenia at 0 min, GLP2: a group with glucopenia at 16 min.

and it was greater than those in the CONT group (28.13±3.83 ng/300 g body wt). However, there was no significant difference in the amounts of glucagon secreted during this later phase among three groups.

Fig. 1 shows a dynamics of insulin secretion from the pancreata of CNS-intact rats. In all three groups, the first and second phases of insulin secretion were observed, which is a typical biphasic secretory pattern. A peak of insulin secretion during the first secretory phase was seen at around 3 min or 4 min in both of the CONT and GLP2 groups. Insulin secretion rate of the GLP1 was the lowest although there was no statistically signifycant difference among groups, and a peak around 4 min was a little bit blunted.

Fig. 2 shows a dynamics of glucagon secretion from the pancreata of CNS-intact rats. In the GLP1 group, a transient spike of glucagon appeared almost immediately, and was followed by the second spike which was characterized by a steep decline. The maximal glucagon secretory rate of the second spike (at 8~9 min) in the GLP1 group was about 2.50 times greater than that in the CONT group. In the GLP2 group, the pancreas seems to secrete about 1.42 times more glucagon than that in the CONT (Table 2), although there was no significant difference between these two groups.

DISCUSSIONS

Insulin and glucagon secretion in this study showes a biphasic pattern, which is similar to that observed in other study reported by Curry et al.[8]. The glucopenic stimulatory effect on glucagon secretion was evident around the second spike of the secretory profile (Fig. 2). The glucagon secretory profiles in both CONT and GLP1 groups show a monotonic decline after 15 min. However, the GLP2 group shows a relatively flat profile between 15 min and 30 min. This behavior may be attributed to the stimulatory effect of glucopenia on the glucagon secretion. In a study with pigs, Karlsson et al.[16] reported that the peripheral glucopenia elevated the plasma glucagon, but exocrine pancreatic secretion level remained the same. Their preparation differs from ours and the plasma glucagon level could be influenced by a release of glucoregulatory factors in response to glucopenia such as epinephrine and norepinephrine[7]. Thus,

their results[16] may not reflect the direct effect of glucopenia on glucagon secretion from the pancreas.

In situ brain-pancreas perfusion used in this study allows that a direct influence of the cephalic glucopenia on islet cell insulin and glucagon secretion since the CNS-innervation of the pancreas is intact, but the pancreatic vasculature is isolated and thus devoid of any secondary humoral and hormonal regulation. A glucagon response to the glucopenia seems to be mediated by neuro-stimulation since a study has reported that a response to glucopenia is abolished by either ganglionic, nicotinic, or muscarinic antagonists and is partially inhibited by adrenergic antagonists[14]. It was suggested a possible mechanism that is largely cholinergic in its efferent pathway[14]. Neuroglucopenia is considered as a physiological stress that may causes metabolic alterations, such as hyperglycemia[22]. Recently, Molina et al.[18] has reported that the hyperglycemia by the neuroglucopenic stress may be caused by the enhanced rate of hepatic glucose production and increased hepatic uptake of gluconeogenic precursors. On the other hand, it has been reported that an glucopenic in peripheral tissues in dogs also increased glucagon level[10], which agrees to our results.

In conclusion, the method used in this study was able to determine the direct effect of cephalic glucopenia on insulin and glucagon secretion from the pancreas without any humoral influences on secretory process. Our results show that the cephalic glucopenia stimulates the glucagon secretion, especially during the early period of perfusion.

Acknowledgement

This work was supported by a grant from Inje University, 1999.

REFERENCES

1) Ahren B and Taborshy Jr GJ (1986): The mechanism of vagal nerve stimulation of glucagon and insulin secretion in the dog. *Endocrinology*, **118:** 1551-1556.

2) Ahren B, Veith RC and Taborshy Jr GJ (1987): Sympathetic nerve stimulation versus pancreatic norepinephrine infusion in the dog: effects on basal release of insulin and glucagon. *Endocrinology*, **121:** 323-331.

3) Bloom SR, Edwards AV and Vaughan NJA (1973): The role of the sympathetic innervation in the control of plasma glucagon concentration in the calf. *J Physiol*, **233:** 457-466.

4) Bloom SR, Vaughan NJA and Russell RCG (1974): Vagal control of glucagon release in man. *The Lancet* Sep, **7:** 546-549.

5) Bonner-Weir S (1991): Anatomy of the islet of Langerhans. pp 15-27, *In* Samols E (ed), "Endocrine Pancreas". Raven Press, New York.

6) Brodows RG, Pi-Sunyer FX and Campbell RG (1973): Neural control of counter-regulatory events during glucopenia in man. *J Clin Invest*, **52:** 1814-1844.

7) Cryer PE. Glucose homeostasis and hypoglycemia. in Wilson JD and Foster DW (1985): Textbook of Endocrinology, 7th Ed, pp 989-1017, W.B. Saunders Co. Philadelphia.

8) Curry DL, Morris JG and Rogers QR (1982): Dynamics of insulin and glucagon secretion by the isolated perfused cat pancreas. *Comp Biochem Physiol*, **72:** 333-338.

9) Desbuquois B and Aurbach GD (1971): Use of polyethylene glycol to separate free and anti-bound peptide hormone in radioimmunoassay. *J Clin Endocrinol*, **33:** 732-738.

10) Frihman LA and Nagai K (1976): Central nervous system-mediated stimulation of glucagon secretion in the dog following 2-deoxyglucose. *Metabolism*, **25:** Suppl 1, 1449-1452.

11) Goodman LS and Gilman A (1980): The Phamacological Basis of Therapeutics. pp 345-382, 8th Ed., New York Pergamon Press, NY.

12) Hall LW (1971): Wright's Veterinary Anesthesia and Analgesia, pp 171-183, 7th Ed., The Williams and Wilkins Company, Baltimore, MD.

13) Havel P, Akpan JO, Curry DL, Stern JS, Gingerich RL and Ahren B (1993): Autonomic control of pancreatic polypetide and glucagon secretion during neuroglucopenia and hypoglycemia in mice. *Am J Physiol*, **265:** (*Regulatory Integrative Comp Physiol* 34): R246-R254.

14) Karlsson S and Ahren B (1987): Inhibition of 2-deoxy-glucose-induced glucagon secretion by muscarinic and α-adrenoreceptor blockade in the mouse. *Diabetes Research and Clinical Practice*, **3:** 239-242.

15) Karlsson S and Ahren B (1991): Contribution of adrenergic nerves and the adrenals to 2-deoxy-D-glucose-induced insulin and glucagon secretion in the mouse. *Int J Pancreatol*, **10:** 207-215.

16) Karlsson S, Pierzynowski SG, Westrom BR, Thaela MJ, Ahren B and Karlsson BW (1995): Stimulation of endocrine, but not exocrine, pancreatic secretion during 2-deoxy-D-glucose-induced neuglucopenia in the conscious pig. *Pancreas*, **11:** 271-275.

17) Miller RE (1981): Pancreatic neuroendocrinology: peripheral neural mechanism in the regulation of the islets of Langerhans. *Endocr Rev*, **2:** 471-494.

18) Molina PE, Williams P and Abumrad NN (1997): Histaminergic contribution to the metabolic effects of neuroglucopenia. *Am J Physiol*, **272** (6 Pt 2): R1918-1924.

19) Oda S, Hagino A, Ohneda A, Sasaki Y and Tsuda T (1988): Adrenergic modulation of pancreatic glucagon and insulin secretion in sheep. *Am J Physiol*, **254:** (*Regulatory Integrative Comp Physiol* 23): R518-R523.

20) Palmer JP, Werner PL, Hollander P and Ensinck JW (1979): Evaluation of the control of glucagon secretion by the parasympathetic nervous system in man. *Metabolism*, **28:** 549-552.

21) Palmer JP, Henry DP and Benson JW (1991): Glucagon response to hypoglycemia in sympathetomized man. *J Clin Invest*, **57:** 522-525.

22) Pascoe WS, Smythe GA and Storlien LH (1989): 2-deoxy-D-glucose-induced hyperglycemia: role for direct sympathetic nervous system activation of liver glucose output. *Brain Research*, **505:** 23-28.

23) Unger RH, Eisentraut AM, McCall MS, Madison LL, Sims K, Timm L and Patman L (1961): Glucagon antibodies and an immunoassay for glucagon. *J Clin Invest*, **40:** 1280-1289.

=국문초록=

중추신경이 온전한 쥐의 Cephalic Glucopenia가 인슐린과 글루카곤 분비에 미치는 영향

인제대학교 의생명공학대학 임상병리학과

최 현 주†

췌장에서 분비하는 인슐린과 글루카곤의 자극-분비 coupling 과정은 주로 혈당 농도와 중추신경계에 의하여 조절되어진다. 본 연구는 頭部에 포도당이 결핍되었을 때에 중추신경계가 췌장에서 인슐린과 글루카곤이 분비되는 패턴을 Sprague-Dawley 흰쥐를 대상으로 하여 살펴보았으며, 실험 방법은 *in situ* 뇌-췌장 관류법을 이용하였다. 관류액은 100 mg/dL glucose와 20 mM arginine를 포함한 Krebs-Ringer 완충액 (pH 7.4)으로 하였으며, 95% O_2-5% CO_2 가스를 계속적으로 주입시키면서 5 ml/min의 속도로 30분간 정주하였다. 대조군은 cephalic glucopenia가 일어나지 않는 군으로 하였고, 실험군은 두 군으로 나누어서 GLP1군은 cephalic glucopenia가 0분에 일어나도록 하였고, GLP2군은 16분에 일어나도록 하였다. 문맥으로 유출되는 췌장의 effluent액에서 인슐린과 글루카곤 농도를 RIA법으로 측정하였고 호르몬의 분비 속도를 산출하여 분비동태 양상을 분석하였다. 결과에서 인슐린 분비량은 GLP1군에서 가장 낮아서 cephalic glucopenia에 의하여 다소 감소하는 경향이었으나, 세 군간에 통계적으로 유의적인 차이는 없었다. 인슐린의 분비동태 양상을 살펴보면 이봉성의 정규 양상을 보였으나, GLP1군에서 첫 번째 peak (4 min)가 다소 둔화되는 현상을 보였다. 글루카곤의 분비동태 양상도 이봉성의 정규 양상을 보였으며, 특히 GLP1군에 있어서 0~15분간의 글루카곤 분비량은 cephalic glucopenia에 의하여 유의성 있게 ($p<0.05$) 증가하였다. GLP2군에 있어서 글루카곤 분비량은 관류 후 15~30분 사이에 증가하는 경향을 볼 수 있었으나 통계적인 유의성은 없었다. 따라서 頭部의 포도당 결핍은 글루카곤의 분비를 증가시키는 것으로 나타났고, 이러한 현상은 특히 관류의 early period에서 현저하였다.

[대한의생명과학회지 6(4): 229-235, 2000년 12월]

†별책 요청 저자