J Tokyo Wom Med Univ
77 (9・10) 501～506 (2007)

# Effect of Endogenous Insulin on Bicarbonate and Amylase Secretion Stimulated by Intraduodenal Infusion of Oleic Acid in Rats

**Yukiko TAKAYAMA, Kyoko SHIMIZU** and **Keiko SHIRATORI**

Department of Gastroenterology, Tokyo Women's Medical University, School of Medicine

(Accepted Aug. 20, 2007)

[Background] It has been well known that islet hormones reach the exocrine pancreas via an islet-acinar portal system. We previously reported that endogenous insulin potentiated amylase secretion stimulated by exogenous and endogenous cholecystokinin (CCK) in rats. The aim of the present study was to investigate the role of endogenous insulin on secretion of bicarbonate as well as amylase in rats. [Methods] The pure pancreatic juice was collected by cannulation of the pancreatic duct in anesthetized rats. Pancreatic exocrine secretion was stimulated by intraduodenal infusion of oleic acid with or without intravenous infusion of glucose. After 1 hour basal period, pancreatic juice was collected for 1 hour and the pancreatic juice volume, bicarbonate and amylase output were measured as pancreatic exocrine secretion. Serum insulin and glucose levels were also measured. [Results] Intraduodenal administration of oleic acid without glucose infusion significantly increased juice volume, bicarbonate and amylase output, comparing to the control. Intravenous infusion of glucose resulted in dose-dependent increase of oleic acid-stimulated juice volume, bicarbonate and amylase output as well as serum insulin levels. [Conclusion] Endogenous insulin potentiated not only amylase but also bicarbonate secretion induced by oleic acid in rats. Therefore, insulin physiologically regulates the duct and acinar cell functions of exocrine pancreas.

**Key words**: bicarbonate secretion, insulin, secretin, cholecystokinin

## Introduction

It has been well known that exocrine pancreas receives a large part of blood draining the islet, and thus islet hormones reach the exocrine pancreas in high concentrations through islet-acinar portal system[1)~3)]. Insulin deficiency impairs pancreatic exocrine function in patients with type I diabetes mellitus[4)5)] and insulin-deficient diabetic animal models[6)~8)]. Insulin therapy and anti-diabetic agents such as thiazolidine derivatives recover the impairment of exocrine function in diabetic patients and animal models[4)~9)]. Thus, insulin is thought to be an important hormone to regulate pancreatic exocrine function. We have previously reported that cholecystokinin (CCK)-stimulated amylase secretion is potentiated by glucose-induced endogenous insulin[10)]. Many investigators have focused on the role of insulin on acinar cell function, however, little is known whether bicarbonate secretion as duct cell function is affected by endogenous insulin. Lee et al[6)~11)] reported that pancreatic exocrine secretion stimulated by physiological doses of CCK and secretin was augmented by exogenous insulin infusion.

The aim of the present study was to evaluate the effect of endogenous insulin on duct and acinar cell function evoked by endogenous release of secretin and CCK induced by intraduodenal administration of oleic acid[12)].

## Materials and Methods

### Animal preparation

Male Wistar rats, weighing 250-300 g, were used in this study. The Committee on Use and Care of Animals at the Tokyo Women's Medical University approved all studies. The rats were fasted for at least 18 hours with free access to water, and then anesthetized with urethane (50% w/v, 0.5 ml/100 g

body weight i.m.) 1 hour before surgery. After placing a midline abdominal incision, the pylorus was ligated to prevent gastric juice from entering into the duodenum, and the common bile duct was ligated proximal to the pancreas below the hilum of the liver. A polyethylene tube (OD 0.9 mm, ID 0.5 mm; Natsume Seisakujo, Tokyo, Japan) was inserted into the bile duct above the ligature, and bile was diverted into the duodenum throughout the experiment. An identical polyethylene tube was inserted into the pancreatic duct at its entrance into the duodenum. A polyethylene cannula (OD 3.4 mm, ID 2.5 mm) was advanced from the stomach into the duodenum via the pylorus for intraduodenal administration of oleic acid. The jugular vein was cannulated with a polyethylene tube (OD 0.8 mm, ID 0.5 mm) for intravenously infusion of glucose, and 0.15 M NaCl solution was infused intravenously at the rate of 1 ml/h with an infusion pump. The rats were kept warm at a constant temperature of 38℃ by heating pads under the body.

**Experimental design**

Experiment 1: Effect of intraduodenal infusion of oleic acid on pancreatic secretion

Sixteen rats prepared by the surgical procedure described above were used in this study. A 0.1 M oleic acid (Nakarai Tesque Inc., Tokyo, Japan) suspension was prepared in 0.15 M NaCl solution and emulsified by Tween 80 (KANTO Chemical 10. INC., Tokyo, Japan), and adjusted to a final pH of 6.5 with 1 M NaOH. The suspended oleic acid solution was administrated intraduodenally at the rate of 1 ml/h with an infusion pump for 1 hour in nine rats. In control experiments, Tween 80 suspension adjusted to same concentration with 0.15 M NaCl solution was infused intraduodenally instead of oleic acid in seven rats.

Experiment 2: Effect of intravenous infusion of glucose on pancreatic secretion stimulated by intraduodenal infusion of oleic acid

Thirty seven rats prepared by the same surgical procedure were used. Glucose (0.25 and 0.5 g/kg/h) was infused intravenously for 2 hours starting at the beginning of basal period. After 1 hour basal period, oleic acid solution was infused intraduodenally at the rate of 1 ml/h for 1 hour. In control experiments, 0.15 M NaCl solution was infused intravenously instead of glucose. Rats were randomly divided into three groups of 14 each in two different dose of glucose (0.25 and 0.5 g/kg/h) and 9 in control.

Blood sample were collected from the tail vein every hour for measurement of glucose. At the end of the experiment, blood was collected from the aorta to determine insulin concentration and centrifuged at 4℃, 3,000 rpm for 30 min, and then stored at −20℃ for future radioimmunoassay of insulin.

**Determinations**

Pancreatic juice was collected continuously by connecting the pancreatic duct cannula to glass micropipettes having a capacity of 0.69 μl/mm tube length (Drummond Scientific Company, Broomall, PA., USA). Volume was determined every hour by measuring the length of the columns of pancreatic juice in the micropipette. After measuring the volume of pancreatic juice, the bicarbonate and amylase concentrations were determined. Bicarbonate concentration of pancreatic juice was determined immediately on the same testing day using a Corning 96, carbon dioxide analyzer (Corning, Essex, UK). The amylase concentration of pancreatic juice was determined by a chromogenic method using blue starch polymer (Pharmacia Diagnostics, Uppsala, Sweden). The 1 hour outputs of bicarbonate and amylase were calculated by multiplying the concentration of each component by the volume of pancreatic juice. The concentration of serum insulin was determined with an insulin radioimmunoassay kit (INCSTAR Corporation, Stillwater, Minn., USA), and the concentration of blood glucose was measured immediately by using hexokinase.

**Statistical Analysis**

All values are expressed as means ± standard error (SE). Unpaired Student's t-test or analysis of variance (analyzed by Stat View, SAS Institute Inc. U.S.A.) were used to evaluate the statistical significance of differences between the control and experimental groups. A probability value of $p<0.05$ was considered statistically significant.

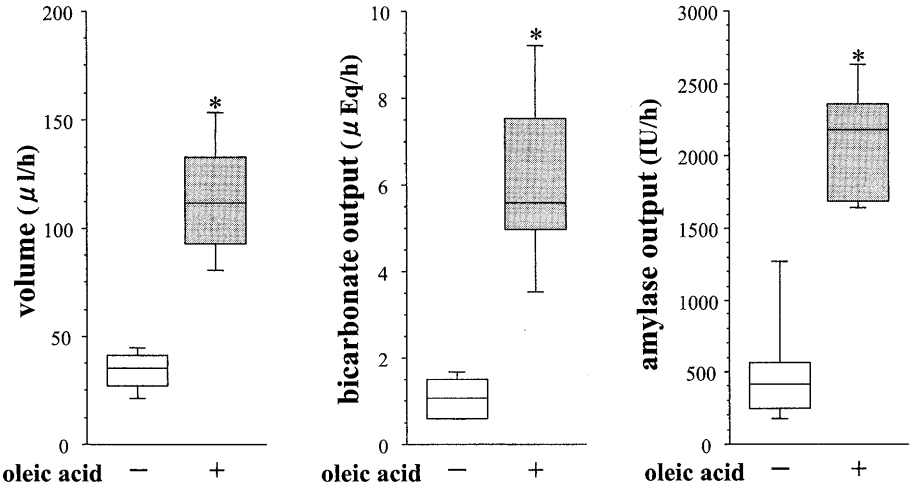


**Fig. 1** Effect of intraduodenal administration of oleic acid on pancreatic juice volume, bicarbonate, and amylase output
Bars represents means ± SE. Intraduodenal administration of oleic acid (□) significantly increased pancreatic juice volume, bicarbonate output and amylase output, comparing to the control (▨) ( * $p < 0.001$).

**Table** Serum insulin and glucose levels in response to intravenous infusion of glucose (0.25 and 0.5 g/kg/h)

| | Glucose (mg/dl) | | Insulin (ng/ml) |
|---|---|---|---|
| | 1 hr | 2 hrs | 2 hrs |
| Control (Saline) | 199.0 ± 8.6 | 231.6 ± 12.1 | 4.96 ± 0.69 |
| Glucose | | | |
| 0.25 g/kg/h | 260.6 ± 28.2 | 345.5 ± 10.4 ** | 6.95 ± 0.50 * |
| 0.5 g/kg/h | 342.2 ± 22.1 *## | 482.7 ± 9.0 *###### | 9.70 ± 0.48 *### |

* $p < 0.05$, ** $p < 0.001$ vs control.
# $p < 0.05$, ## $p < 0.005$, ### $p < 0.001$ vs 0.25 g/kg/h.

## Results

Intraduodenal administration of oleic acid significantly increased pancreatic juice volume (115.9 ± 9.5 μl/h), bicarbonate output (6.1 ± 0.7 μEq/h) and amylase output (2075.6 ± 134.1 IU/h), comparing to the control (33.3 ± 3.3 μl/h, 1.1 ± 0.2 μEq/h, and 574.6 ± 159.1 IU/h, respectively) ($p<0.001$) (Fig. 1).

Serum insulin and glucose levels were dose-dependently increased in response to intravenous infusion of glucose (0.25 and 0.5 g/kg/h) (Table). Intravenous infusion of glucose in a dose of 0.25 g/kg/h failed to potentiate pancreatic secretion stimulated by oleic acid. Intravenous infusion of glucose in a dose of 0.5 g/kg/h resulted in a further increase in oleic-acid stimulated pancreatic juice volume (161.3 ± 10.6 μl/h), bicarbonate output (8.5 ± 0.6 μEq/h) and amylase output (3,150.0 ± 287.1 IU/h) ($p<0.05$ vs each base-line) (Fig. 2).

## Discussion

In the present study, intravenous infusion of glucose significantly augmented both bicarbonate and enzyme secretion in response to intraduodenal infusion of oleic acid. Since serum concentration of insulin was dose-dependently increased by intravenous infusion of glucose, endogenous insulin may play a role on potentiation of pancreatic exocrine secretion stimulated by oleic acid. Intraduodenal administration of oleic acid is already known to stimulate both secretin and CCK release in rats, suggesting that secretin-stimulated bicarbonate secretion as well as CCK-stimulated enzyme secretion is augmented by endogenous insulin release. Since a small amount of

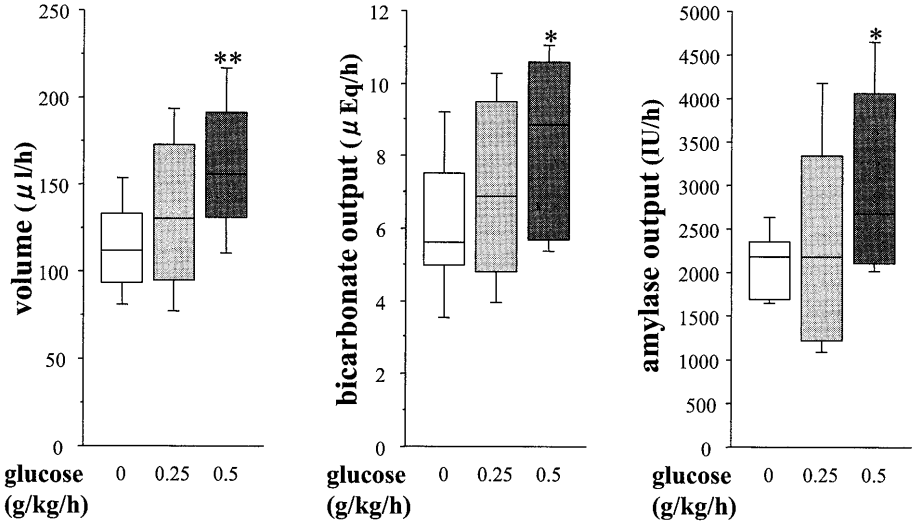


**Fig. 2** Correlation between serum glucose or insulin levels and doses of intravenous infusion of glucose

Bars represents means ± SE. Control (□), glucose in a dose of 0.25 g/kg/h (▨), glucose in a dose of 0.5 g/kg/h (■), ( * $p < 0.05$, * * $p < 0.01$).

Intravenous infusion of glucose in a dose of 0.25 g/kg/h failed to potentiate pancreatic secretion stimulated by oleic acid. Intravenous infusion of glucose in a dose of 0.5 g/kg/h resulted in a significant increase in oleic-acid stimulated pancreatic juice volume ($p < 0.01$ vs base-line), bicarbonate output and amylase output ($p < 0.05$ vs base-line).

insulin release did not affect oleic acid-induced pancreatic secretion, this proves that the process depends on insulin concentration.

We have previously reported that endogenous release of insulin potentiated pancreatic enzyme secretion stimulated by exogenous CCK or intraduodenal infusion of casein that is known to be a potent CCK secretagogue[10]. Lee et al[6] reported that in conscious rats immunoneutralization of circulating insulin with the anti-insulin serum resulted in a complete suppression of postprandial pancreatic exocrine secretion. Furthermore, the anti-insulin serum completely blocked the secretin- and CCK-induced pancreatic secretion, indicating that the physiological doses of secretin and CCK can exert their action on the exocrine pancreas only in the presence of circulating insulin. In totally isolated perfused pancreas of rats and dogs, immunoneutralization of circulating insulin paradoxically increased somatostatin and pancreatic polypeptide (PP) in portal effluent[12)13)]. Addition of anti-somatostatin and anti-PP serum reversed the bicarbonate and amylase secretion suppressed by anti-insulin serum. Other group also reported that exogenous somatostatin inhibited the interaction of endogenous insulin and CCK[14]. These results suggest that a local action of insulin plays an important role on the stimulatory action of secretin and CCK on bicarbonate and enzyme secretion, and a part of its action is mediated by the supression of endogenous somatostatin and PP that are known to inhibit the pancreatic exocrine secretion. Although we could not measure the somatostatin and PP concentration in the portal vein, it is speculated that the stimulatory effect of endogenous insulin on oleic acid-stimulated pancreatic exocrine secretion may be mediated by the inhibition of local release of somatostatin and PP.

Endogenous release of insulin can reach acinar and ductal cells of the pancreas through the insuloacinar portal system and interstitium, therefore, insulin seems to have a direct action on the exocrine pancreas. It has been reported that insulin enhances the acinar cell protein, RNA, and DNA synthesis[15)~17)], upregulates amylase gene expression[18)], and downregulates its own receptors[19)]. Furthermore, insulin stimulates pancreatic exocrine secre-

tion mediated by increasing $Na^+$, $K^+$-ATPase activity[20]. Although it is unclear whether insulin directly enhances bicarbonate secretion on ductal cells, it may be possible that insulin stimulates oleic acid-stimulated bicarbonate secretion on duct cells as well as acinar cells.

In conclusion, endogenous insulin has a potentiated action on pancreatic bicarbonate and amylase secretion stimulated by intraduodenal administration of oleic acid, and insuloacinar axis plays an important role in the regulation of pancreatic secretion in response to ingestion of a meal.

### References


1) **Bonner-Weir S, Orci L**: New perspective on the microvasculature of the islets of Langerhans in the rat. Diabetes **31**: 883–889, 1978
2) **Ohtani O, Ushiki T, Kanazawa H et al**: Microcirculation of the pancreas in the rat and rabbit with special reference to the insulo-acinar portal system and emissary vein of the islet. Arch Histol Jpn **49**: 45–60, 1986
3) **Von Schönfeld J, Goebell H, Müller MK**: The islet-acinar axis of the pancreas. Int J Pancreatol **16**: 131–40, 1994
4) **Frier BM, Faber OK, Binder C et al**: The effect of residual insulin secretion on exocrine pancreatic function in juvenile-onset diabetes mellitus. Diabetologia **14**: 301–304, 1978
5) **Chey WY, Shay H, Schuman CR**: External pancreatic secretion in diabetes mellitus. Ann Intern Med **59**: 812–821, 1996
6) **Lee KY, Xhou L, Ren XS et al**: An important role of endogenous insulin on exocrine pancreatic secretion in rats. Am J Physiol **258**: G268–G274, 1990
7) **Lee KY, Lee YL, Kim CD et al**: Mechanism of action of insulin on pancreatic exocrine secretion in perfused rat pancreas. Am J Physiol **267**: G207–G212, 1994
8) **Lee KY, Kwon HY, Park HS et al**: The role of insulin in the interaction of secretin and cholecystokinin in exocrine secretion of the isolated perfused rat pancreas. Pancreas **12**: 58–63, 1996
9) **Shimizu K, Shiratori K, Hayashi N et al**: Effect of troglitazone on exocrine pancreas in rats with streptozotocin-induced diabetes mellitus. Pancreas **21**: 421–426, 2000
10) **Iwabe C, Shiratori K, Shimizu K et al**: Role of endogenous insulin in pancreatic secretion in rats. Pancreatology **1**: 300–305, 2001
11) **Saito A, Williams JA, Kanno T**: Potentiating of cholecystokinin-induced exocrine secretion by both exogenous and endogenous insulin in isolated and perfused rat pancreas. J Clin Invest **65**: 777–782, 1980
12) **Shiratori K, Watanabe S, Takeuchi T et al**: Role of secretin and cholecystokinin in oleic acid-stimulated pancreatic secretion in rats. J Gastroenterol **25**: 104–111, 1990
13) **Lee KY, Krusch D, Zhou I et al**: Effect of endogenous insulin on pancreatic exocrine secretion in perfused dog pancreas. Pancreas **11**: 190–195, 1995
14) **Garry DJ, Garry MG, Williams JA et al**: Effects of islets hormones on amylase secretion and localization of somatostatin binding sites. Am J Physiol **256**: G897–G904, 1989
15) **Okabayashi Y, Mossner J, Longsdon CD et al**: Insulin and other stimulants have nonparallel translational effects on protein synthesis. Diabetes **36**: 1054–1060, 1987
16) **Korc M, Iwamoto Y, Sankaran H et al**: Insulin action in pancreatic acini from streptozotocin-treated rats. Stimulation of protein synthesis. Am J Physiol **240**: G56–G62, 1981
17) **Mossner J, Logsdon CD, Williams JA et al**: Insulin via its own receptor regulates growth and amylase synthesis in pancreatic acinar AR42J cells. Diabetes **35**: 891–897, 1985
18) **Korc M, Owerbach D, Quinto C et al**: Pancreatic islet-acinar cell interaction: Amylase messenger RNA levels are determined by insulin. Science **213**: 351–355, 1981
19) **Williams JA, Bailey AC, Peissler M et al**: Insulin regulation of sugar transport in isolated pancreatic acini from diabetic mice. Diabetes **31**: 674–682, 1982
20) **Matsushita K, Okabayashi Y, Koide M et al**: Potentiating effect of insulin on exocrine secretory function in isolated rat pancreatic acini. Gastroenterology **106**: 200–206, 1994

# ラットにおけるオレイン酸十二指腸内投与による重炭酸およびアミラーゼ分泌刺激に対する内因性インスリンの増強効果についての検討

東京女子医科大学消化器内科
高山（タカヤマ）　敬子（ユキコ）・清水（シミズ）　京子（キョウコ）・白鳥（シラトリ）　敬子（ケイコ）

〔目的〕膵ホルモンは，islet-acinar portal system を介して膵外分泌腺に灌流することが知られている．以前我々は，ラットにおける内因性・外因性コレシストキニン（CCK）刺激による膵外分泌が，内因性インスリンによって増強することを報告した．そこで今回，ラットにおける膵重炭酸分泌に対する内因性インスリンの効果について検討した．

〔対象と方法〕麻酔下のラットで純粋膵液を採取し，十二指腸にオレイン酸（pH 6.5，0.1mmol）と，対照として生理食塩水，Tween 80 を 1 時間持続投与した．また，経静脈的にグルコース 0.25，0.5 g/kg/h，対照として生理食塩水を併用投与した．膵液の液量，重炭酸濃度，アミラーゼ濃度，およびグルコース負荷前後の血糖，実験終了時の血中インスリン濃度を測定した．

〔結果〕オレイン酸の十二指腸内投与により，膵液量（μl/h），重炭酸分泌量（μEq/h），アミラーゼ分泌量（IU/h）はいずれも対照群に比べ有意に増加した．血糖・血中インスリン濃度は，グルコース負荷により，用量と相関して上昇した．グルコース負荷群でもオレイン酸刺激下の膵液量，重炭酸分泌量，アミラーゼ分泌量のいずれもグルコースの用量依存性に増加し，オレイン酸単独投与群に比べグルコース 0.5 g/kg/h 投与群でいずれも有意に分泌反応が増強された．

〔結語〕高用量ブドウ糖負荷は内因性インスリンにより，分泌の増加を介してオレイン酸の腸管内投与による膵液・重炭酸・アミラーゼ分泌量を有意に増強した．内因性インスリンは，腺房細胞のみならず導管系に対しても分泌増強効果を示すことから，islet-acinar portal system におけるインスリン分泌は，膵外分泌調節に重要であると考えられる．