Estrogen increases Glucose-Induced Insulin Secretion from Mouse Pancreatic Islets cultured in a Prolonged High Glucose Condition

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Background: *It is known that males are more susceptible to develop type 2 diabetes than females. Estrogen has a protective effect on pancreatic islet against toxic agent such as amyloid. The role of estrogen in protection pancreatic islet against high glucose is still unknown.*
Objective: *Administration of estrogen in an ovariectomised animal shows a protective effect against type 2 diabetes. The present study aimed to determine the direct effect of estrogen on the islet function after prolonged culture in high glucose.*
Material and Method: *Estrogen (10^{-5} M in ethanol) was co-cultured with mouse pancreatic islets in normal glucose medium (11.1 mM) for 3 hours or with normal and high glucose medium (40 mM) for 10 days.*
Results: *Estrogen increased glucose-induced insulin secretion in islet culture in normal glucose medium for both 3-hour and 10-day culture. Prolonged exposure of pancreatic islet to high glucose generated impaired glucose-induced insulin secretion, which was partially abrogated by the presence of 10^{-5} M estrogen.*
Conclusion: *These results indicated a direct effect of estrogen on improving insulin secretion from mouse pancreatic islets that has been impaired by prolonged exposure to high glucose.*

Keywords: *Insulin secretion, High glucose culture, Estrogen, Mouse pancreatic islet, In vitro study*

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Type 2 diabetes is characterized by insulin resistance and impaired glucose-induced insulin secretion. Defect in insulin secretion is known to cause hyperglycemia in type 2 diabetes[1]. Prolonged hyperglycemia produces diabetic complications and β-cell death[2,3]. Sex differences have been noticed to affect development of this disease[4-7]. Males are more susceptible to develop type 2 diabetes than females[8,9].

The effect of estrogen on glucose metabolism is still debated. Estrogen-replacement studies showed both improvement[10-13] and impairment[14] in glucose metabolism. Several studies suggested that estrogen has a protective effect against the risk of type 2 diabetes[10-12,15], which occurs more frequently in postmenopausal women[6,16,17]. Results from the previous studies are difficult to interpret since they involve *in vivo* studies, which are complicated by several uncontrolled factors.

Non-classical plasma membrane estrogen receptors (ncmER) have been found on β-cells[18]. Estrogen stimulates insulin secretion by binding to its receptor at the cell membrane and activating guanylyl cyclase[19]. Increased amount of cGMP activates protein kinase G (PKG), which phosphorylates K_{ATP} -sensitive channel and generates insulin secretion[19]. On the other hand, estrogen has been demonstrated to increase insulin secretion via up regulating of insulin gene expression[20]. Taken together, insulinotropical effect of estrogen may be by both non-transcriptional and transcriptional actions.

Estrogen also has a protective effect on pancreatic islet against toxic agents such as amyloid-[15] and cytokine-induced cell death[21]. Furthermore, es-

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trogen has been shown to prevent glucose toxicity in retinal pericytes[22]. The effect of estrogen on glucose-induced insulin secretion in prolonged cell culture in high glucose media is still unknown. Therefore, the authors investigated the direct effect of estrogen on impaired glucose-induced insulin secretion in pancreatic islets that have been in prolonged culture in high glucose media.

Material and Method

Animal

Male ICR outbred 8-12 week old mice were purchased from the National Laboratory Animal Center, Mahidol University, Bangkok, Thailand.

Mouse pancreatic islet isolation and culture

Mice were fed with rat chow and housed in a temperature-controlled room at 25-30°C with 12 h light/dark cycle for 3-7 days before being sacrificed by CO_2 gas inhalation. An incision was made from the end of the sternum to the end of the ribs on both sides. The mouse was then placed under a stereomicroscope and the liver and duodenum were displaced to uncover the bile duct. Pancreatic islets were isolated by collagenase digestion using a modified method of Lacy & Kostianovsky[23]and Gotoh[24]. The bile duct was clamped off at its entrance to the duodenum and 2.5 ml of collagenase-P solution was injected into the common bile duct through a bent 30G needle. The distended pancreas was removed and kept in a 50 ml conical tube on ice. Ten ml of warm RPMI media were added into tubes containing the collagenase-P filled pancreases, which were incubated at 37 C for 20 minutes. Digested pancreases were filtered through a filter (mesh sized of 600 μm) and washed twice in cold RPMI medium. The isolation of the islets was accomplished by using a histopaque gradient and hand picking under a stereomicroscope. Isolated islets were cultured in RPMI 1640 medium supplemented with 10% fetal calf serum, 100 U/ml penicillin, and 100 μg/ml streptomycin at 37 C in humidified air containing 5%CO_2 for 24 hours.

Pancreatic islets were then incubated in 11.1 mM glucose RPMI 1640 containing 10^{-5} M 17β- estradiol or the same amount of absolute ethanol for 3 hours. Insulin secretion assay was then performed in the presence of 2.8, 5.6, 10, 15, and 20 mM glucose.

Pancreatic islets were also incubated in RPMI 1640 containing 11.1 mM or 40 mM glucose with or without 10^{-5} M 17β- estradiol for 10 days. The culture medium was changed every other day. On day11, insulin secretion assay was performed in the presence of 2.8 or 20 mM glucose.

Measurement of insulin secreted into culture medium

Islets were washed twice in Krebs Ringer bicarbonate buffer (KRB) (111 mM NaCl, 4.8 mM KCl, 2.3 $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 25 mM $NaHCO_3$), containing 10 mM Hepes, 2.8 mM glucose, 0.2%BSA fraction V (Sigma Chemical Co.). Pre-incubation of islets was conducted in KRB for 90 min at 37 C. Triplicate batches of five pre-incubated islets were transferred to borosilicate tubes containing 1 ml of KRB supplemented with desired glucose concentration. These triplicate batches of islets were then incubated for 60 min at 37 C and the supernatant was collected after centrifugation at 2,000 rpm, at 4 C for 5 min and stored at -20 C till measurement of insulin using a rat specific commercial radioimmunoassay (Pharmacia).

Statistical analysis

Comparison of insulin secretion between two different glucose concentrations was performed using unpaired Student's *t test*. Significant difference was set at $p < 0.05$. Data are presented as mean $\pm$ SEM.

Results

Short-term effect of estrogen on glucose-induced insulin secretion

Cultured islets in the presence of 10^{-5} M 17-β estradiol for 3 hours in 11.1 mM glucose significantly showed higher insulin response to 5.6, 10, 15, and 20 mM glucose compared to control (Fig. 1A). Fold insulin stimulations compared with 2.8 mM glucose in islets co-culture with 10^{-5} M 17-β estradiol augmented with increase in glucose concentration, but plateaud at 15 mM glucose (Fig. 1B).

Long-term effect of estrogen on glucose-induced insulin secretion

Cultured islets in normal glucose medium (11.1 mM) showed insulin secretion stimulated by 2.8 and 20 mM glucose similar to those of 3-hours (Table 1). Fold stimulation compared to 2.8 mM glucose between short-term (4.59 ± 0.46) and long-term (6.42 ± 1.05) was not statistically different. However, glucose-induced insulin secretion by 20 mM glucose was impaired in the 10-days cultured islet in high glucose (40 mM) compared to normal glucose medium (11.1 mM) (Fig. 2). Islets in high glucose medium were co-cultured for 10 days with 10^{-5} M 17-β estradiol had similar glucose-induced (20 mM) insulin secretion to that of cells

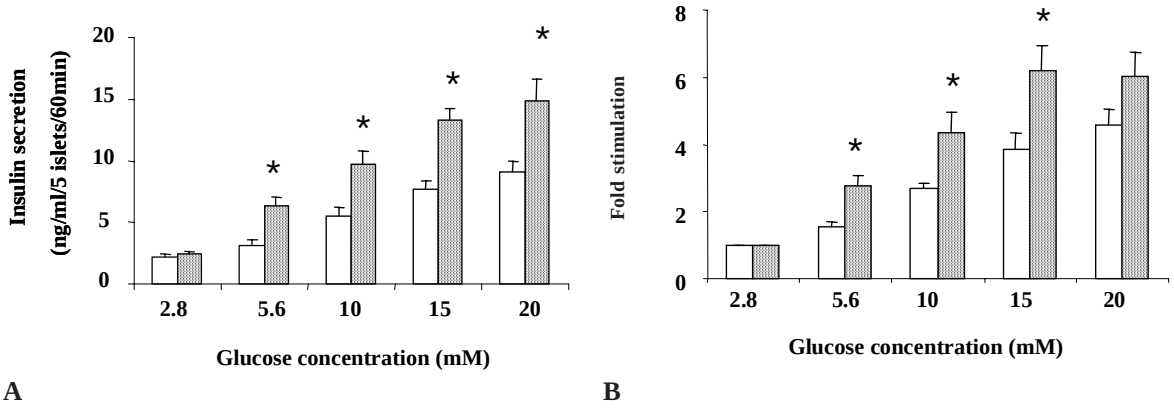

FIg. 1 Insulin secretion from isolated islets cultured for 3 hours in normal glucose medium in the the absence (☐) and presence (▨) of 10^{-5} M 17β-estradiol
A. Insulin secretion, B. Fold stimulation compare to 2.8 mM glucose. Values are expressed as mean ± SEM (n = 6-9)
* $p < 0.05$, relative to absence of 10^{-5} M 17β-estradiol

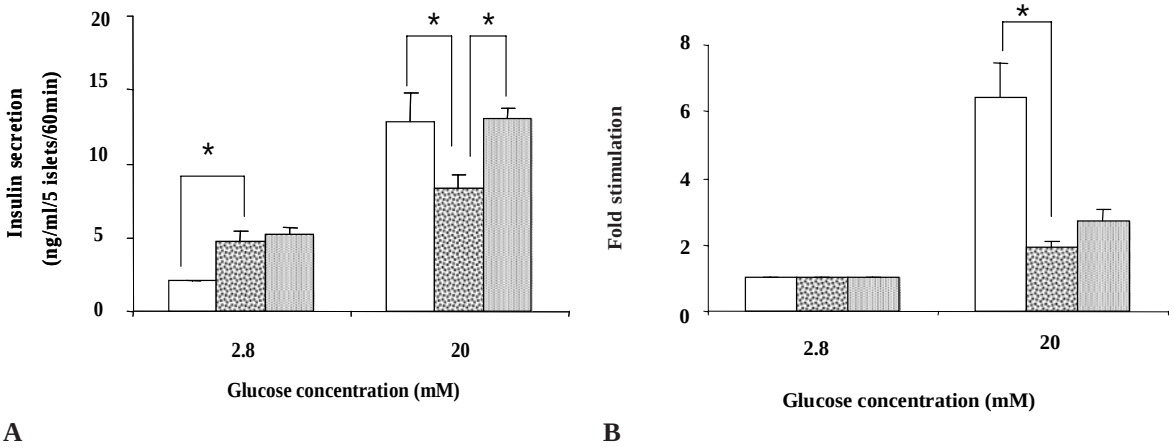

Fig. 2 Insulin secretion from isolated islets cultured for 10 days in normal glucose medium (☐) and high glucose medium in the absence (▨) or presence (▨) of 10^{-5} M 17 β-estradiol
A. Insulin secretion, B. Fold stimulation compared to 2.8 mM glucose. Values are expressed as mean ± SEM (n = 6)
* $p < 0.05$

Table 1. Insulin secretion of isolated islets cultured in normal glucose medium for 3 hours and 10 days. Values are expressed as mean ± SEM (n = 6-9)

Glucose concentration (mM)	Insulin secretion (ng/ml/5 islets/60 mins)	
	3 hours	10 days
2.8	2.10 ± 0.30	2.04 ± 0.07
20	9.13 ± 0.83	12.88 ± 1.88

cultured in normal glucose without estradiol (Fig. 2). This indicated that 10^{-5} M 17-β estradiol was able to improve islet-cell function that had been impaired by high glucose during long-term culture.

Discussion

Culturing islets with 10^{-5} M estrogen increased insulin secretion both in short (3hours) and long-term (10 days) exposure. It has been shown previously that the insulinotropical effect of estrogen is generated by both its non-transcriptional and transcriptional

actions[18-20]. Non-transcriptional effect occurred rapidly (2-3 min) after adding estrogen into the media[25]. In the present study, estrogen was added during a 3-hour culture in normal media, thus, the insulinotropical action of estrogen shown in the present study was likely to occur by an up regulation of the insulin gene.

Insulin secretion and fold stimulation by 2.8 mM glucose of mouse pancreatic islets cultured for 10 days in high glucose medium (40 mM) was significantly lower than from those cultured in normal glucose medium (11.1 mM). Decrease in insulin secretion was due to both high basal insulin secretion at 2.8 mM glucose and reduced insulin secretion at 20 mM glucose. This indicated an impaired β-cell function in high glucose culture. High basal insulin secretion is a characteristic of impaired insulin secretion shown in several studies[26-29]. Long-term over activation of β-cells was shown to produce high basal insulin secretion[30] followed by a decrease of insulin pool in β-cells[29,31]. In addition, insulin gene expression was decreased by chronic high glucose exposure in previous experiments[32,33]. Thus, chronic high glucose exposure caused β-cell dysfunction both insulin production and secretion.

Estrogen significantly increased glucose-induced insulin secretion from islets that had been cultured for 10 days in high glucose medium. To the authors' knowledge, this is the first report that estrogen is able to directly effect on the improvement of β-cell function from long-term exposure to high glucose environment. Estrogen appears to contain an antioxidant capacity and prevents the damage of several tissues such as brain[34,35] and vascular[36,37] from oxidative stress. Antioxidants have been shown to protect pancreatic islets from glucotoxicity[38]. Thus, it is possible that the protective effect of estrogen was achieved by estrogen-enhancing insulin gene expression and/or other mechanisms. These conjectures require further investigation.

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เอสโตรเจนเพิ่มการหลั่งอินซูลินจากกลุ่มเซลล์ตับอ่อนที่เลี้ยงในระดับน้ำตาลสูง

สุวัฒณี คุปติวุฒิ, น้ำอ้อย เสมประเสริฐ, สุพรพิมพ์ เจียสกุล

***ภูมิหลัง**: เพศชายเสี่ยงต่อการเกิดโรคเบาหวานชนิดที่ 2 มากกว่าเพศหญิง นอกจากนี้เอสโตรเจนสามารถป้องกันกลุ่มเซลล์ตับอ่อนต่อสารพิษ เช่น อะมัยลอยด์ได้ เอสโตรเจนมีผลป้องกันกลุ่มเซลล์ตับอ่อนต่อระดับน้ำตาลสูงหรือไม่*
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***วัสดุและวิธีการ**: กลุ่มเซลล์ตับอ่อนเลี้ยงในน้ำเลี้ยงที่มีเอสโตรเจนร่วมกับน้ำตาลระดับปกติ เป็นเวลานาน 3 ชั่วโมง และร่วมกับน้ำเลี้ยงที่มีน้ำตาลระดับปกติและสูงเป็นเวลา 10 วัน*
***ผลการศึกษา**: เอสโตรเจนเพิ่มการหลั่งอินซูลินเมื่อเติมลงในน้ำเลี้ยงที่มีระดับน้ำตาลปกติทั้งที่เวลา 3 ชั่วโมง และ 10 วัน เซลล์ตับอ่อนหลั่งอินซูลินลดลงเมื่อเลี้ยงในน้ำเลี้ยงที่มีระดับน้ำตาลสูงเป็นเวลา 10 วัน แต่เมื่อเลี้ยงในระดับน้ำตาลสูงร่วมกับเอสโตรเจนเป็นเวลา 10 วันทำให้อินซูลินกลับหลั่งเพิ่มขึ้น*
***สรุป**: การศึกษาครั้งนี้แสดงให้เห็นว่าเอสโตรเจนช่วยป้องกันกลุ่มเซลล์ตับอ่อนซึ่งทำงานด้อยลงเมื่ออยู่ในน้ำเลี้ยงที่มีระดับน้ำตาลสูงมีผลให้กลับหลั่งอินซูลินดีขึ้น*