In Vitro Study of Insulin Secretion from Mouse Pancreatic Islets-Siriraj Experiences

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Abstract: Pancreatic islet isolation and culture technique are tools for direct investigation of the effects of substances on insulin secretion. Glucose is a well known insulin stimulating substance from the pancreatic islet. This study aims to demonstrate the first success (in Thailand) in islet isolation with intact insulin secretion from a mouse pancreas using collagenase P enzyme and histopaque separation. Isolated islets were cultured in RPMI 1640 for 24 hours before undergoing a glucose stimulation test. Glucose at five different concentrations (2.8, 5.6, 10, 15 and 20 mM) was used to demonstrate the dose-dependent response curve. Insulin secretion when stimulated with 5.6, 10, 15 and 20 mM glucose was higher than with 2.8 mM basal glucose concentration. Insulin secretion increased about 1.7 to 3.5 fold from a basal level of 2.8 mM glucose without any difference in insulin content at any glucose concentrations used. To our knowledge, these data demonstrate the first success in mouse pancreatic islet isolation and culture in Thailand. This technique can be used as a tool for further investigation of the in vitro effects of substances such as plants or new drugs on insulin secretion.

Key words: Glucose-stimulated insulin secretion, mouse pancreatic islet isolation, in vitro study, cell culture

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Reference:
57: 14-18.
INTRODUCTION

Endocrine cells in the pancreas are a group of numerous cells called the islet of Langerhans. These islets are scattered throughout the exocrine portion of the pancreas. Pancreatic islets are composed of at least four different types of endocrine cells. These different types of cells produce separated pancreatic hormones. Two pancreatic hormones that play an important role in tightly control blood glucose are insulin and glucagon. Insufficient insulin secretion causes hyperglycemia in diabetic patient. Although it is believed that genetics and the environment play major roles in the development of impaired insulin secretion, the definite cause of defects in pancreatic islet function is still unknown.

The technique of pancreatic islet isolation has been developing for over three decades. Pancreatic islet can be isolated by the combination of mechanical and enzymatic approaches. Isolation of mouse pancreatic islets is less difficult than isolation from bigger mammals. There are several applications for pancreatic islet isolation such as for studying pathophysiology of glucotoxicity, lipotoxicity, and the effect of insulin stimulating agents. In spite of the meticulous procedure, we report the first successful setting-up method of mouse pancreatic islet isolation at Siriraj. This established procedure offers a possibility for further studies of in vitro insulin secretion for various purposes, especially for the pathophysiology and treatment of both types of diabetes in Thailand.

MATERIALS AND METHODS

Source of tissues

Male 8-12 week-old ICR outbred 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, at 12h light/dark cycle for 3-7 days before being sacrificed by CO₂ gas inhalation for 2 minutes. Pancreatic islets were isolated by collagenase digestion using a modified method of Lacy & Kostianovsky and Gotoh et al. Mice were killed and an incision was made from the end of the sternum to the end of the ribs on both sides. The mouse was placed under a stereomicroscope and the liver and duodenum were displaced to uncover the bile duct. 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 was added into the tube containing the collagenase-P filled pancreases. The pancreases were digested at 37°C incubation for 20 minutes. Digested pancreases were filtered (mesh sized 600 µm) and washed twice in cold RPMI media. 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 media supplemented with 10% fetal calf serum, 100 U/ml penicillin, and 100 µg/ml streptomycin at 37°C in humidified air 5%CO₂. The culture medium was changed every other day.

Measurement of insulin secretion in culture medium

Islets were washed twice in Krebs Ringer Bicarbonate Buffer (KRB) (111 mM NaCl, 4.8 mM KCl, 2.3 CaCl₂, 1.2 mM MgSO₄, 1.2 mM KH₂PO₄, 25 mM NaHCO₃), 10 mM Heps, 2.8 mM glucose, 0.2%BSA fraction V (Sigma Chemical Co.). Preincubation of islets was done in the same buffer for 90 min at 37°C. Triplicate batches of 5 preincubated islets were transferred to borosilicate tubes containing 1 ml of KRB supplemented with glucose concentrations at 2.8, 5.6, 10, 15 and 20 mM, respectively. These three batches of islets in different glucose concentrations were then further incubated for 60 min at 37°C and the medium was collected after centrifugation at 2,000 rpm, at 4°C for 5 min. The culture medium were stored at -20°C until the measurement of insulin with a rat specific commercial radioimmunoassay (RIA) kit (Pharmacia) was performed.
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Statistical analyses
The comparisons of insulin secretion or insulin content between the two different glucose concentrations in the culture media were performed using paired Student’s *t* test. Significant difference was set at *P*< 0.05. Insulin data in all figures are presented as mean ± SEM.

RESULTS

Insulin secretion from isolated islets after 24h incubation in RPMI 1640
To determine islet function *in vitro*, isolated islets were cultured in RPMI 1640 for 24 hours. The insulin secretory capacity over a period of 60 min was assessed by increasing glucose concentrations in KRB. The results showed that isolated islets from ICR mice significantly secreted higher insulin in response to 10,15 and 20 mM glucose concentrations compared to a 2.8 mM glucose concentration (basal condition) (Table 1). Insulin secretion at stimulated conditions were 1.7 to 3.5 fold higher than the basal condition (2.8 mM glucose) (Figure 1).

Table 1. Insulin secretion and glucose concentrations after culture in RPMI 1640.

<table>
<thead>
<tr>
<th>Glucose concentration (mM)</th>
<th>Insulin secretion (ng/ml/5 islets/60mins)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.8</td>
<td>5 ± 0.8</td>
<td>-</td>
</tr>
<tr>
<td>5.6</td>
<td>8.6 ± 1.7</td>
<td>0.072</td>
</tr>
<tr>
<td>10</td>
<td>10.8 ± 2.0</td>
<td>0.023</td>
</tr>
<tr>
<td>15</td>
<td>13.5 ± 2.4</td>
<td>0.007</td>
</tr>
<tr>
<td>20</td>
<td>14.2 ± 2.6</td>
<td>0.005</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (n=5-6).

DISCUSSION
The technique of pancreatic islet isolation has been developed for the purpose of pancreatic islet transplantation into diabetic patients. The isolation techniques progressed from an earlier standard chopped tissue technique to the present intraductal collagenase standard method. The intraductal method gives more numbers of islets than the standard chopped technique. Using the technique described in this paper, the isolated islet obtained appears morphologically intact. In this study we used histopaque for separation islet from exocrine tissue instead of ficoll and bovine serum gradients. The latter two reagents were more expensive, required long preparation and entailed a finicky process. Our experiment demonstrated that islet survival with...
histopaque separation was similar to the ficoll gradient that has been used by previous investigators\textsuperscript{15,16}. Thus, histopaque was more convenient and economical than the ficoll gradient. Also, the ability to release insulin in response to glucose stimulation has been shown to be preserved following culture in a RPMI 1640 medium supplemented with 10% fetal calf serum\textsuperscript{17,18}. This isolation method provides a tool for obtaining a large quantity of viable islets. It may be used to assess the insulin secretory function as well as for pancreatic islet transplantation in rodents. Also, the opportunity to study the physiology of intact islet function \textit{in vitro} has been achieved.

This study demonstrates that mouse pancreatic islets are able to be isolated and cultured within our own facilities at Siriraj Hospital. Insulin secretion from these islets was shown to increase as the glucose concentrations in the media was increased. More than 3 fold higher insulin secretion was obtained when stimulated with 20 mM glucose compared to 2.8 mM glucose. Our results were similar to other previous studies using rodent islets\textsuperscript{19-21}. However, insulin contents were not different when stimulated with 2.8 or 20 mM glucose since insulin secretion from an islet is normally less than 2 % in a stimulated condition\textsuperscript{22}. Thus, similarity in insulin content suggested that islet size was not different in each glucose concentrations and increased insulin secretion is not due to different islet size.

In conclusion, we demonstrated the success in isolation of mouse pancreatic islet at Siriraj Hospital that has not been reported before in Thailand. The dose-dependent response curve of insulin secretion shows the intact functional abilities of isolated islets and culture. Further experiments to understand more about the defects in insulin secretion in diabetes and the study of insulin secretory capability by various biological substances will be carried on.

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REFERENCES

13. Lake SP, Chamberlain J, Bassett PD, et al. Successful reversal of diabetes in nude rats by transplantation of


