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Observation

Sustained glucose-stimulated insulin secretion in mouse islets is not culture-dependent

To the Editor: The process of sustained glucose-evoked insulin secretion has been extensively studied, although the molecular mechanisms implicated are still poorly understood. Biphasic insulin release, and more specifically its associated second phase, is a subject of long-standing and intensive research [1]. In this context, it is intriguing that the dynamics of insulin secretion in a mouse model differ, depending on which of two widely used experimental preparations are employed. Using in situ pancreatic perfusion, glucose-stimulated insulin release is essentially monophasic, with a very weak second phase following the 4- to 5-min first phase [2, 3]. In contrast, once isolated, pancreatic islets exhibit sustained secretory responses to glucose in a perifusion protocol. How the mouse beta cell can switch from the transient (in situ) to the sustained (in vitro) secretory mode is unknown. Investigators commonly keep the islets in culture between the isolation procedure and perifusion experiments. This suggests the possibility that culture conditions might induce changes in gene expression and/or enzyme activity, thereby modifying the machinery of metabolism-secretion coupling. For instance, glucokinase activity is induced in mouse islets cultured at high glucose [4]. Such culture-dependent changes are supposed to modify glucose metabolism, thereby promoting signal generation implicated in the sustained phase of insulin release. Here, we questioned the effects of culture periods on the secretory profiles of isolated mouse islets in response to glucose stimulation.

The kinetics of glucose-stimulated insulin release were measured in mouse islets at different time periods following isolation. Pancreatic islets were isolated by collagenase digestion from male BALB/c mice weighing 25 to 30 g [5]. For Day 0, freshly isolated islets were used 1 to 2 h after isolation. For cultured islets, isolated mouse islets were cultured free-floating in RPMI-1640 medium (11.1 mmol/l glucose) with 5% fetal calf serum for 3 and 7 days before the secretion assay. Islet perifusions were carried out as described [5] using 15 to 30 hand-picked islets per chamber of 250-µl volume maintained by thermostat at 37 °C (Brandel, Gaithersburg, Md., USA). The flux was set at 0.5 ml/min and after a 20-min washing period at basal glucose, fractions were collected every minute with glucose changes as detailed below. Insulin levels were determined by radioimmunoassay using rat insulin as standard [5]. Insulin secretion was expressed as the fraction of insulin collected every minute, normalised per 10 islets (ng insulin/10 islets).

Mouse islets were perifused with Krebs-Ringer bicarbonate HEPES buffer at basal 2.8 mmol/l glucose for 15 min. They were then stimulated with 11 mmol/l glucose for 30 min and subsequently returned to 2.8 mmol/l glucose (Fig. 1). Freshly isolated islets responded to the first 15 min of glucose stimulation with a 6.6-fold ($p<0.005$) increase in insulin secretion rate over basal release, as assessed by area under the curve calculations. These Day 0 islets exhibited 10.1-fold ($p<0.001$) sustained increases in secretory responses for the last 15 min of stimulation versus insulin release at 2.8 mmol/l glucose. Islets maintained in culture for 3 days showed a similar secretory profile to freshly isolated islets, although the amplitude of the secretory response was weaker. Indeed, the area under the curve calculated over the 30-min period of glucose stimulation was lower by 42% than for fresh islets (24.6±0.1 vs 42.1± 3.8 ng insulin released per 10 islets over 30 min, $p<0.01$). Extending mouse islet culture to 7 days did not modify the kinetics of glucose-stimulated insulin secretion. As compared to Day 3, islets cultured for 7 days exhibited lower amplitudes of insulin secretion at 11 mmol/l glucose than freshly isolated islets (–53%, $p<0.01$). This might be explained by a reduction of insulin content in cultured islets, either at Day 3 (–77%, $p<0.001$) or Day 7 (–52%, $p<0.01$), versus fresh islets (290± 43 ng insulin/islet). Surprisingly, both freshly isolated (Day 0) and cultured (Days 3 and 7) islets responded to glucose stimulation with a sustained phase of insulin secretion. This should be compared to the in situ perfused pancreas, where a transient first phase is followed by a weak second phase [2, 3]. Therefore, we conclude that mouse islets possess the intrinsic capacity of exhibiting sustained glucose-stimulated insulin secretion.

Our data show that culture of isolated mouse islets does not modify the pattern of secretion kinetics. The procedure alone of islet isolation from the in situ pancreas confers robust and prolonged secretory responses to glucose. This suggests that rather than inducing expression changes in beta cells during culture, isolating islets relieves some in situ inhibition. Sympathetic neurotransmitters such as neuropeptide Y (NPY) and galanin are known to inhibit insulin secretion [6]. Activation of corresponding receptors on beta cells would inhibit the cyclic-AMP-dependent protein kinase A (PKA) pathway. In fact, the PKA pathway has been implicated in sustained phase of insulin secretion [7]. Moreover, NPY has been shown in a mouse model to inhibit specifically glucose-stimulated insulin secretion, while calcium-mobilising agent stimulation was not affected [8]. Therefore, it can be hypothesised that inhibitory mechanisms of insulin secretion, present in the mouse in in situ perfused pancreas are absent once islets are isolated.

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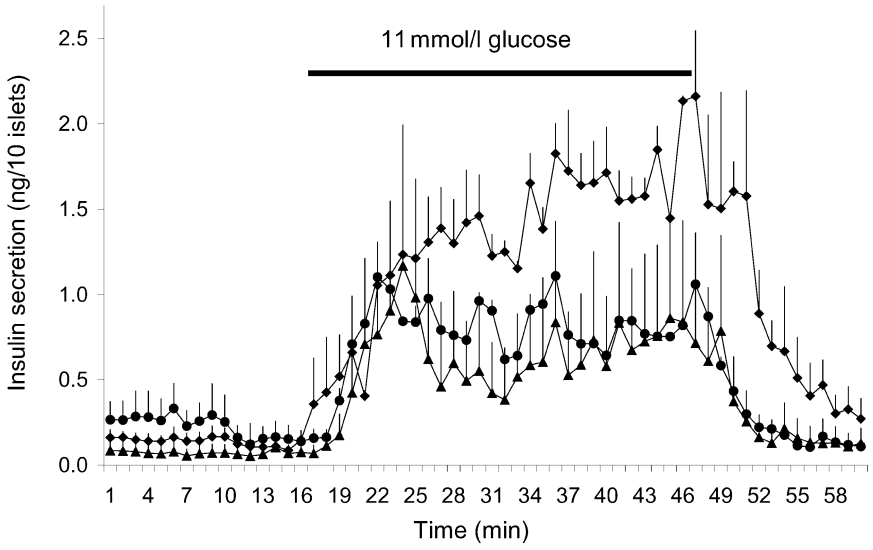

Fig. 1. Kinetics of glucose-stimulated insulin secretion of freshly isolated and cultured mouse islets. Mouse islets were perifused directly after isolation (Day 0, ◆) or after culture periods of 3 (●) or 7 (▲) days. Perifusion took place at basal 2.8 mmol/l glucose for 15 min, followed by stimulation with 11 mmol/l glucose for 30 min, returning subsequently to 2.8 mmol/l glucose. Values are means + SD of 3 perifusion chambers representing one of three independent experiments

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