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Technical Note

A cell perifusion system of isolated rat islets for studies on rapid insulin-glucose dynamics

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ABSTRACT

We have developed a cell perifusion system of isolated rat islets, to improve the accuracy of controlling the medium content and the stability of the medium flow. This new system presents the following characteristics: (1) There is always a consistent flow in the perifusion chamber and only half of its volume is altered when switching the medium. (2) The medium containing test substances can be prepared and held ready in very close proximity to islets. The test glucose media are introduced to the islets immediately after a switch. (3) The environment of the islets could be repeatedly changed from one state to another in a rigorous stepwise manner. The system can provid an accurate switch of glucose concentrations both in time and quantity without introducing pressure disturbances, which makes a suitable tool for studies on rapid insulin–glucose dynamics. In experiments of perifused rat islets, our results further confirmed the observation that the amplitudes of oscillatory insulin secretions were magnified when glucose levels increased but their periods were unaffected. In addition, insulin secretions instantly increased in response to the sudden increase of glucose levels, but gradually decreased in response to the sudden decrease of glucose levels. © 1997 *IPEM Published by Elsevier Science Ltd*

Keywords: Perifusion, pancreatic islet, insulin dynamics

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1. INTRODUCTION

The perifusion of pancreatic islets is useful in gaining knowledge about hormone secretions. For biomedical applications, the perifusion information from a bunch of islets is very important in modeling and system design[1,2]. In the implantable bioartificial pancreas [3–5], more than ten thousand healthy isolated islets were transplanted into a sophisticated implantable device acting like a perifusion chamber for diabetes therapy. In a perifusion system, a group of islets are isolated from the pancreas and cultured long-term in a current of a medium, which makes it possible to monitor changes in the pattern and the rates of hormone secretion from islets in response to acute stimulation. However, instrumental artifacts such as the disturbance of liquid pressure due to switching the perifusion flows and the time delay or irregular overshoots due to the alterations of medium content will significantly blur the dynamics of insulin secretions. We have developed a perifusion system to improve the control accuracy (in quantity and timing) for altering the medium content and the stability of medium flow, liquid pressure and temperature. This new system provides a suitable tool to investigate the rapid insulin–glucose dynamics. In the experiments on perifused rat islets, our results further confirmed that the amplitudes of oscillatory insulin secretions are magnified when glucose concentrations are increased, but their periods are unaffected. In addition, we found that insulin secretion instantly increased but gradually decreased in response to sudden increase and decrease of glucose levels, respectively.

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2. MATERIALS AND METHODS

2.1. Isolation of rat islets

Pancreatic islets were isolated by using the collagenase technique of Gotoh *et al.*[6] Male Wistar rats (NTU, Taipei, ROC) were anesthetized and 10 ml of collagenase solution was injected into the common bile duct to distend the pancreas. This was excised and placed in a water bath at 37°C for digestion. After a series of washing steps, the islets were separated from other tissue fragments by centrifugation on a discontinuous Ficoll (Sigma, MO, USA) gradient in Hank's balanced salt solution with specific gravities of 1.080 and 1.110. About 500 clean islets were obtained and cultured in RPMI-1640 medium containing 100 mg/dl glucose (GIBCOBRL, supplemented with 0.2% sodium bicarbonate, 1.5% HEPES, 10% newborn calf serum, 0.2% penicillin–streptomycin, and 0.1% amphotericin B) at 37°C, in a CO_2 incubator for 24 h to ensure the complete recovery of islets.

2.2. Perifusion system

A schematic diagram of our perifusion system is shown in *Figure 1*. About 100 similar size islets were hand-picked under the microscope and placed into a perifusion chamber, which comprised a barrel of 1 ml plastic syringe (TERUMO, Tokyo, Japan) and two layers of nylon net with 10 μm pores (Pharmacia, Uppsala, Sweden) placed at the bottom to lightly hold the islets (*Figure 2*). To control the exact time of introducing test substance into the chamber and to reduce the disturbances of medium flow, the method of tubing arrangement from Watanabe and Orth[7] was modified. Several parallel tubes (mainline, sideline 1, 2, 3) were arranged as shown in *Figure 1*. Each of the sidelines was connected with a three-way stopcock and a 21-gauge needle. All tubes were driven by a multi-channel peristaltic pump at an individual flow rate of 180 μl/min. Under the basal perifusion condition, one sideline (side 1) which contained the same medium as the mainline was inserted through the upper port of the chamber and its three-way stopcock was opened, which resulted in a total flow rate of 360 μl/min. Another selected sideline (side 2), primed with test solution and its three-way stopcock closed, was also inserted into the chamber until the tip of the needle nearly reached near the

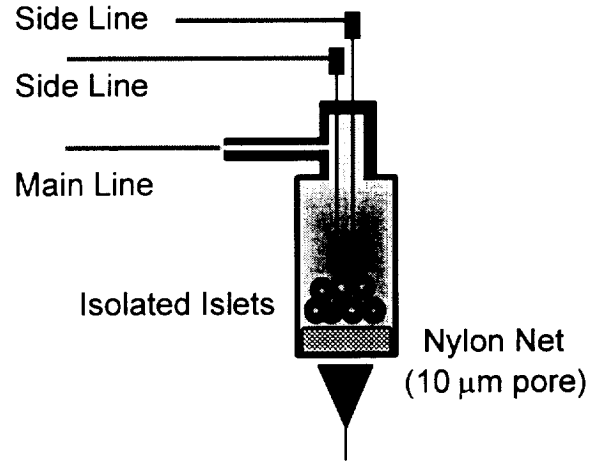

Figure 2 Schematic representation of the perifusion chamber. It was composed of a barrel of 1 ml plastic syringe and two layers of nylon net with 10 μm pores were placed in the bottom. Each sideline primed with test solution and connected with a 21-gauge needle was inserted into the chamber until the tip of the needle was near the islets.

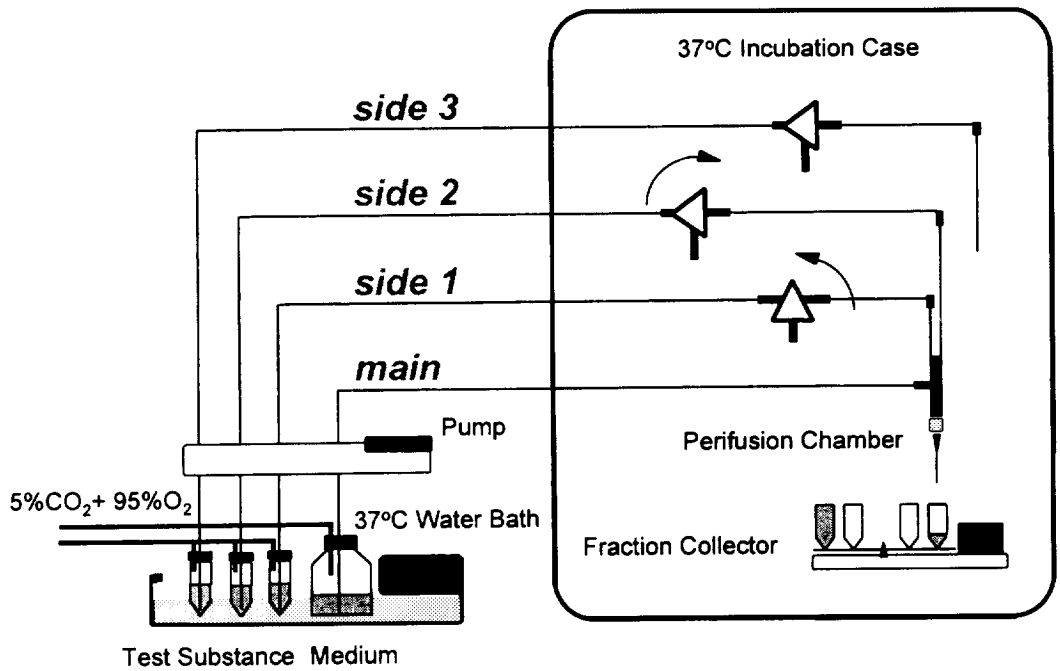

Figure 1 Schematic representation of the perifusion system. The tube arrangement was adopted from Watanabe and Orth[7] with some modifications. To begin perifusion of test substance, two three-way stopcocks were turned in opposite directions simultaneously. The media in two sidelines were immediately exchanged (side 1→side 2). The procedures could be repeated (side 2→side 3) acting like a relay. Multiple identical sets of tubes and chambers can be performed simultaneously.

islets (*Figure 2*). To switch the media, the two three-way stopcocks were turned simultaneously in opposite directions. Thereby media in two sidelines were immediately exchanged (side 1→side 2). The test substance was added to the vicinity of the islets with half concentrations and nearly no dead space. These procedures could be repeated for another pair of sidelines (side 2→side 3) acting like a relay. Perifusion media were prewarmed in a 37°C water bath and gassed with 5% CO_2–95% O_2. The rest of the system was also operated in a 37°C incubator. The temperature of the system was measured by two sensors (Pt 100 Ω, one in a water bath, another bound to the chamber) and maintained by an electronic controller. Samples were collected in 3-min fractions and were frozen for subsequent analysis. Insulin concentrations in the collected perifusate were determined by radioimmunoassay (RIA)[8] with ^{125}I-labeled human insulin and human standard.

3. RESULTS

3.1. System capability in glucose alteration

Figure 3 demonstrates the system capability in the alterations of glucose concentrations. Media were infused by shutting off the flow of one sideline (100 mg/dl) and simultaneously opening the other sideline sequentially to deliver medium containing glucose of various concentrations (500, 300, 0 mg/dl). Perifusate was immediately measured by glucose analyzer (model 2300 STATPlus, Yellow Springs Instrument, OH, USA). The sampling interval was reduced to 30 s near the switching point. By quickly mixing with medium from the mainline (100 mg/dl), glucose concentration was instantly changed to each expected level (300, 200, 50 mg/dl). The system demonstrated a nearly perfect stepwise function of glucose alteration.

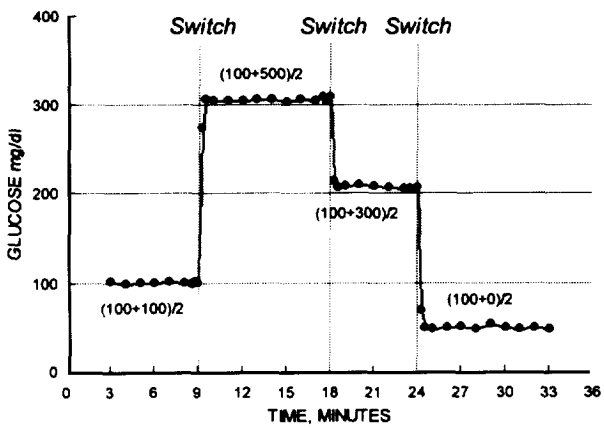

Figure 3 Capability of system in the alterations of glucose concentrations. Sidelines were switched to deliver media containing 100, 500, 300, 0 mg/dl glucose acting like a relay, and quickly mix with medium from mainline (100 mg/dl) to present 100, 300, 200, 50 mg/dl glucose levels in a stepwise function. The sampling interval was reduced to 30 s near the switching point.

3.2. Pressure disturbances due to switching the perifusion flows

Islets from the same rat were perifused (*Figure 4*) in two parallel chambers ("a", "b"). A switch of glucose concentration from 100 to 100 mg/dl was arranged for each chamber to investigate the pressure effects due to flow switches. Different switch procedures were performed in the two chambers, with directly switching through a three-way stopcock on chamber "a" (*Figure 4*A) but with our switching design on chamber "b" (*Figure 4*B). The insulin secretions in chamber "a" were suddenly increased directly after the switch. This violent insulin secretion gradually decreased to a level even lower than that observed before the switch, then returned toward the basal level. Because the marked increase of insulin was not synchronous in the parallel chamber "b", it should not be ascribed to any other artifacts such as temperature or system vibration but pressure disturbance due to switching the flows. This phenomenon was consistent in the four repeated experiments. The average peak value of insulin secretion was 138.7 ± 27.4 μU/ml (n = 4), approx. twice the basal insulin level (58.9 ± 7.9 μU/ml in a glucose level of 100 mg/dl). On the other hand, chamber "b" was unaffected when switching the medium (*Figure 4*B), which was also consistent in all of the four repeated experiments.

3.3. Rapid insulin–glucose dynamics

When islets were continuously perifused with medium containing glucose at concentrations ranging from 100 to 200 then to 300 mg/dl, a spontaneous oscillation of insulin secretion was observed in each glucose concentration interval

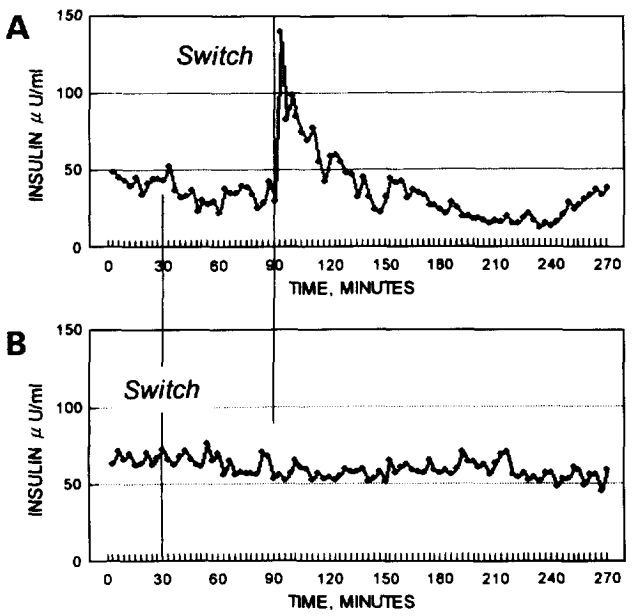

Figure 4 Pressure disturbance on insulin secretions when switching the medium flow. Islets in two parallel chambers (a, b) were perifused with media containing 100 mg/dl glucose throughout the test. Different devices for two chambers are shown on the right side. (A) Chamber "a" directly switched the tubing through a three-way stopcock at the 90 min time point. (B) Chamber "b" was switched at 30 min by our system design.

(*Figure* 5A). In addition, the amplitudes of oscillatory insulin were magnified when glucose concentrations were raised, but their periods were unaffected (approx. 15 min). The average amplitudes of three repeated experiments were 5.2 ± 2.1, 9.3 ± 3.7 and 12.8 ± 3.4 μU/ml with respect to glucose concentration intervals of 100, 200 and 300 mg/dl. In the subsequent experiment, perifusion was performed in three parallel chambers

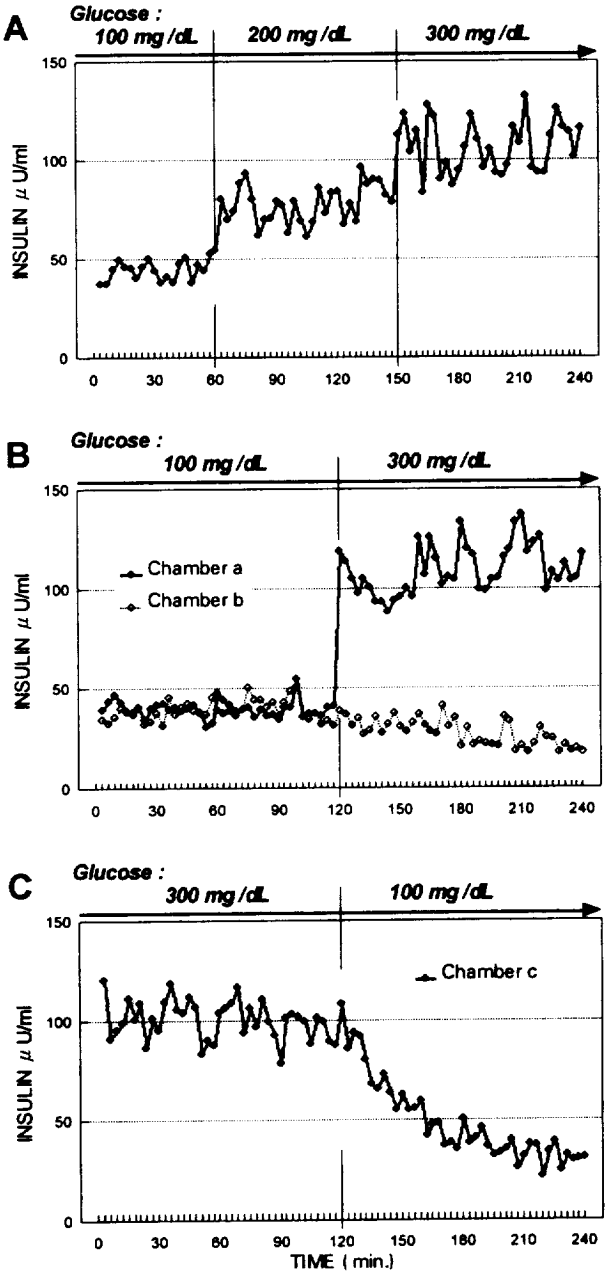

Figure 5 Rapid secretory response of insulin from isolated rat islets. (A) Islets were perifused with medium containing glucose changed from 100→200→300 mg/dl in a stepwise function, a spontaneous oscillation of insulin was observed at each glucose interval. The amplitudes of oscillatory insulin were magnified when glucose concentrations were raised, but their periods were unaffected. In another experiment, islets from one rat were divided into three parallel chambers (a, b, c) and exposed to different glucose alterations individually. (B) Chamber "a" was from 100→300 mg/dl, chamber "b" was from 100→100 mg/dl. (C) Chamber "c" was from 300→100 mg/dl. Note that insulin secretion instantly increased (chamber a), but gradually decreased (chamber c) in response to increased and decreased glucose levels, respectively.

("a", "b", "c"). Islets from the same rat were divided into three groups and exposed to different alterations in glucose levels. Glucose in chamber "a" was increased from 100 to 300 mg/dl (*Figure* 5B), chamber "c" was from 300 to 100 mg/dl (*Figure* 5C), and control chamber "b" was from 100 to 100 mg/dl (dashed line in *Figure* 5B). The disturbance of medium flow was prevented since the insulin secretion in control chamber "b" was unaffected throughout the test. We found that insulin secretion instantly increased (chamber "a") in response to the rigorous step of glucose increase, but gradually decreased (chamber "c") in response to the rigorous step of glucose decrease. In addition, a prominent first-phase insulin secretory peak was observed in chamber "a", which was more marked than that when glucose level was increased stepwise (100→200→300 mg/dl, *Figure* 5A). The foregoing experiments were repeated three times and consistent results were observed.

4. DISCUSSION

A slight pressure change produced due to switching the perifusion flows would induce a violent increase of insulin. Each islet in the experiment had been carefully re-examined (400× microscope) after perifusion, and was confirmed to be in complete integrity. The violent increase of insulin secretion was therefore not due to an extensive leak of insulin from crashed islets. However, not only in the dynamic shape but also in the quantity of increment, the violent increase of insulin was very similar to the well-known first-phase secretory peak of insulin[9]. The secretory insulin response to a sudden increase of glucose is an acute first-phase peak followed by a sustained second phase of increased secretion, which persists for the duration of the high-glucose stimulus. During the first phase of the secretory response, the release of insulin is due to the extrusion of secretory granules already located in the cell web near the cell membrane. During the second phase, the release of insulin depends on the provision of secretory granules transported to the cell web. We believe that the mechanism of the first-phase insulin response might also be induced by the slight pressure change produced due to switching. When islets were slightly pressurized, the secretory granules located near the membrane of B-cells might be extruded and result in a false first-phase secretion. However, the mechanism of second phase with the transportation of secretory granules to the cell web should not be evoked since the glucose level was unchanged throughout the test. This might account for the observation in *Figure* 4(A) that after the pressure-induced secretory peak, insulin secretion did not present a sustained second phase of increase, but gradually decreased then returned toward the basal level. In the present study, the pressure disturbances on insulin secretions have been successfully prevented. As a result of our system design, insulin secretion was unaffected when the perifusion media were switched and glucose concentrations

changed from 100 to 100 mg/dl (*Figure* 4B), which was consistent in all four repeated experiments. The new system presented the following characteristics: (1) There was always a consistent flow of medium in a perifusion chamber and only half of its volume was altered in a switch of the medium flow. Thus pressure disturbances were prevented. (2) The media in the sidelines have been prepared and held ready in very close proximity to islets. They were immediately mixed with the medium from the mainline right after the switch. Accurate control in timing and quantity for altering medium content was achieved. (3) These procedures could be repeated from one sideline to another acting like a relay. The environment of islets therefore could be repeatedly changed in a rigorous step from one state to another. Consequently, our system provides a suitable tool to investigate detailed insulin–glucose dynamics, such as the pulsatile insulin release and the rapid response of insulin to precisely controlled alterations in glucose levels.

Pancreatic islets secrete insulin in an oscillatory fashion[10–12]. It was reported that glucose regulates the amplitudes of the insulin pulses but did not affect their periods[13–15]. This observation was further confirmed by an identical group of islets in the present study. The glucose concentration in the vicinity of the islets was continuously changed from 100 to 200 then to 300 mg/dl in a stepwise manner. Our results show that the amplitudes of the insulin pulses increased in approximate proportion to the glucose level, but that their periods remained unaffected. In addition, the first-phase secretory peaks in both cases when glucose level changed from 100 to 200 mg/dl and from 200 to 300 mg/dl, were less marked than that when glucose changed from 100 to 300 mg/dl. These findings implied that the amplitudes of insulin oscillation might be dose-dependent on the glucose levels, yet the first-phase secretory peaks of insulin might be dose-dependent on the increment of glucose levels. The phenomena lend support to the hypothesis of dose-dependent recruitment of glucose-exposed B-cells[16,17]. Intercellular differences in the threshold for glucose cause different numbers of B-cells to secrete at a specific glucose level. The proportional increase of amplitudes with the stepwise increase of glucose might reflect the fact that more and more B-cells were recruited into an oscillatory state. A summation of all B-cells' synchronously pulsatile secretions would result in an enhanced oscillation with magnified amplitude but with the same periodicity. For the first-phase secretory insulin peaks, the greater increment of the glucose alteration might then reflect that more B-cells were fired to secrete after a switch. In these just-activated B-cells, the secretory granules already located near the cell membranes were extruded to release insulin. Therefore, the more B-cells are just-activated, the stronger the first-phase insulin secretory peak would be induced by the summation of the insulin released from these B-cells. This might account for the fact that the first-phase insulin secretory peak when the glucose level was increased in one step (100→300 mg/dl, *Figure* 5B) was more marked than that when the glucose level was increased stepwise (100→200→300 mg/dl, *Figure* 5A). By comparing two types of glucose alterations, from 100 to 300 mg/dl and from 300 to 100 mg/dl, insulin instantly increased but gradually decreased in response to a sudden increase and decrease of glucose level, respectively. We believe that this intrinsic characteristic of islets might result in a physiological benefit. Insulin secretion instantly increased when glucose suddenly increased. Abundant insulin was introduced to blood to relieve the hyperglycemia as soon as possible. Conversely, when glucose suddenly decreased, the delay in insulin decrease could temporally maintain the transportation of blood glucose into cells in defense against the hypoglycemia.

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