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Insulin Release from the Perifused Isolated Rat Langerhans Islets under a Slow-rise Glucose Stimulation

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The dynamics of insulin release from the perifused rat Langerhans islets was investigated under a slow-rise and square-wave stimulation by glucose.

Every dose response curve were sigmoidal profile, in which the threshold of glucose was shown to be around 4.2 mM. The K_m values of dose response curves were similar each other under a slow-rise and square-wave stimulation by glucose. Since a rise of gradient level from 0.10 to 0.22 mM/min did not alter the dose response curve, it was proved that a profile of insulin release in response to slowly rising glucose was not due to a lag time in insulin release but dependent to an absolute change of glucose level. The stimulation by rapid change of glucose level with 2.80 mM/min of gradient level caused an insulin releasing profile similar to that by the square-wave stimulation.

Consequently, it was suggested that the insulin release under a slow-rise stimulation by glucose at gradient levels around 0.10—0.22 mM/min was more physiologic. This stimulation method is an easily available procedure for preparing accurate dose response relationship between the rate of insulin released and the concentration of glucose.

Keywords——insulin release; dose response curve; slow-rise stimulation; square-wave stimulation; perifusion; pancreatic B cell; glucoreceptor; sigmoid; pancreatic islet of Langerhans

Introduction

Important role of D-glucose as a stimulus for insulin release has been established by *in vitro*[2] and *in vivo*[3] experiments. Recent studies on insulin release using the α and β anomers of D-glucose suggested that the pancreatic B cell may contain glucoreceptors.[4] Accurate dose response relationship of insulin release to glucose concentration over wide range may add important information to clarify the glucoreceptor mechanism involved in insulin secretory responses.

We now examined insulin-releasing profile from the perifused rat Langerhans islets by a slow-rise stimulation by glucose of gradient concentrations and the dose response curve obtained under the above condition.

Materials and Method

Reagents——All reagents used were special grade. D-Glucose, theophylline, NaCl, $CaCl_2 \cdot H_2O$, KH_2PO_4, $MgSO_4 \cdot 7H_2O$ and $NaHCO_3$ were products of Wako Pure Chemical Industries Ltd., Japan. Bovine serum

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albumin (fraction V) was purchased from The Armour Laboratories Ltd., USA and collagenase from Sigma Chemical Co., USA. Insulin RIA kits were purchased from Dainabot Radioisotope Laboratories, Ltd., Japan. Sodium pentobarbital (general anesthetic) was product of Abbot Laboratories, USA.

Isolation of Islets——Pancreatic islets of Langerhans were isolated from overnight fasted male Wistar rats weighing 120—200 g by the method of Lacy and Kostianovsky.[5] In average about 120 islets (diameter 200—250 μ) were obtained from one animal. Islets of comparable sizes collected from three animals were used for a series of experiments.

Incubation Medium——The medium used for all experiments was Krebs-Henseleit bicarbonate buffer (pH 7.35) which was supplemented with 0.5% bovine serum albumin and 1 mM theophyline and equilibrated with a mixed gas of O_2 and CO_2 (95: 5, v/v). The partial pressure of oxygen (pO_2) in the medium was maintained at 114.3—144.5 mmHg under atmosphere of the mixed gas. D-Glucose was added to the medium before use.

Incubation——Ten islets of a comparable sizes were placed in each flask containing 1 ml of the medium with 2.8 mM glucose. After a 30-min control equilibration, the incubation was carried out in the medium supplemented with various glucose concentrations during the period of 150 min at 37° under an atmosphere of the mixed gas.

Measurements——Insulin levels (immunoreactive insulin, IRI) were measured by a double antibody immunoassay and glucose contents by a glucose analyzer (ERA 2001, Beckman Instruments INC.). Partial pressure of oxygen (pO_2) in test medium was determined by a blood gas analyzer (BMS-MK series, Denmark Radiometer, Co., Ltd.).

Perifusion with a Square-wave Stimulation by Glucose——One hundred and twenty islets collected from three rats were perifused in a plastic flow cell maintained at a constant temperature (37°) and the average flow rate of the perifusate was 0.5 ml/min. During the 30-min control period each perifusion system was equilibrated with a medium containing 2.8 mM glucose and then the perifusions were carried out with a square-wave stimulation by glucose at various concentrations. The perifusate was collected by a fraction collector at a 2-min interval.

Perifusion with a Slow-rise Stimulation by Glucose——After a control equilibration for 30 min with a medium containing 2.8 mM glucose, the perifusion was carried out for 150 min under a slow-rise stimulation by glucose with some linear gradient levels which was indicated as GPM (Δ glucose mM/min), from 2.8 mM glucose.

Results

Dynamics of Insulin Release from the Incubated Rat Islets

After a 30-min equilibration with 2.8 mM glucose, incubations were carried out in the media containing 2.8, 5.6, 11.1, 16.7 and 27.8 mM glucose for 150 min, respectively. The amounts of insulin released in response to glucose are shown in Fig. 1. The dose response curve was sigmoid with a K_m of 10.0 mM glucose, showing that insulin release reached a plateau (2245+195 μU/ml/10 islets/150 min, $N=6$, mean±S.E.M.) at about 16.7 mM glucose.

Dynamics of Insulin Release from the Perifused Rat Islets under a Square-wave Stimulation by Glucose at Various Concentrations

After a 30-min control equilibration with a medium containing 2.8 mM glucose, a perifusion was carried out under a square-wave stimulation by glucose at a concentrations of 2.8, 5.6, 11.1, 16.7 and 27.8 mM for 150 min and then the insulin releasing profiles are shown in Fig. 2.

The dynamics of insulin release from the perifused islets caused by glucose ranging from 11.1 mM to 27.8 mM showed rapid increase 10 min after the perifusate arrived at the flow cell, and reached a plateau within 30 min, and afterwards the amounts of insulin release kept constant levels during the period of 120 min.

Insulin release from the rat islets perifused with the medium containing 5.6 mM glucose began 15 min after arrival at the flow cell of perifusate and the amount of insulin release reached a plateau at 30 min, and afterwards gradually declined close to a base line during the period of 120 min. However, an initial peak, the first phase of insulin release, could not observed under any stimulation by glucose with five different concentrations described above.

5) P.E. Lacy and M. Kostianovsky, *Diabetes*, **16**, 35 (1976).

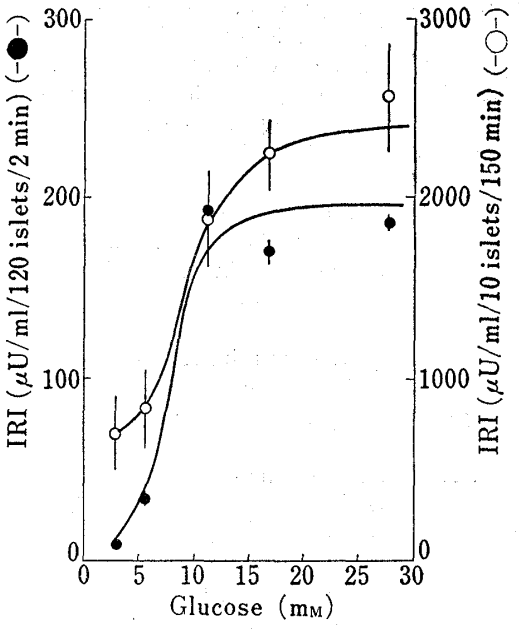

Fig. 1. Dose Response Curves for the Insulin Release from the Perifused and Incubated Rat Islets with Glucose at a Constant Level

Each point represents the mean±S.E.M. The dose response curve with closed circles was derived from the amounts of insulin released in the perifusion system induced by various glucose concentrations (left ordinate). The dose response curve with open circles derived from the amounts of insulin released in the incubation system with various glucose concentrations (right ordinate).

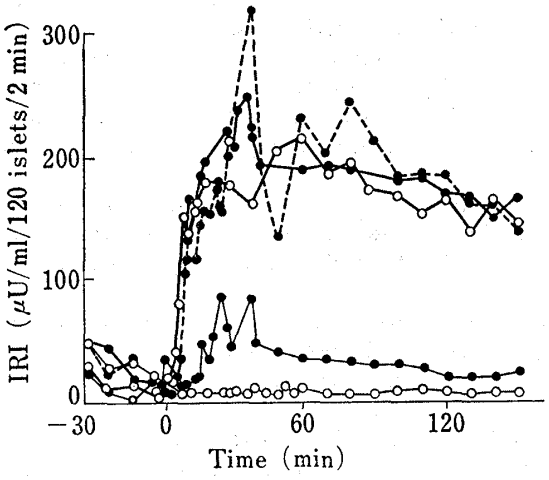

Fig. 2. Dynamics of Insulin Release under a Square-wave Stimulation

Each point represents the mean insulin released from the perifused rat islets under a square-wave stimulation by glucose at the concentrations of 2.8 mM (○—○ $N=2$), 5.6 mM (●—● $N=5$), 11.1 mM (●---● $N=3$), 16.7 mM (○—○ $N=5$) and 27.8 mM (●—● $N=5$).

Release of insulin was also not stimulated with 2.8 mM glucose, and insulin was released at a basal rate (9±1.0 μU/ml/120 islets/2 min, $N=2$, mean±S.E.M.) as shown in Fig. 2.

The dose response curve derived here was sigmoid with a Km value of 7.6 mM glucose and became a plateau (193±8.3 μU/ml/120 islets/2 min, $N=5$, mean±S.E.M.) at about 11.1 mM glucose as shown in Fig. 1.

Dynamics of Insulin Release from the Perifused Rat Islets under a Slow-rise Stimulation by Glucose

After a control equilibration for 30 min with the medium containing 2.8 mM glucose, each perifusion was carried out for 150 min with a slow-rise stimulation by glucose at linear gradient levels, which was indicated as GPM (0.10, 0.22 and 2.80 mM/min) (Fig. 3 A,B,C). As shown in Fig. 3A, insulin release from the perifused rat islets was initially observed at about 4.2 mM glucose attained 20 min after the beginning of the perifusion, and then the insulin release was increased proportionally with the increasing glucose levels untill insulin release reached a plateau at concentration of around 11.1 mM glucose, and the amount of insulin release kept a constant level (180±24 μU/ml/120 islets/2 min, $N=3$, mean±S.E.M.) during the further period of 65 min.

As shown in Fig. 3B, insulin release from the perifused rat islets under a slow-rise glucose stimulation with 0.22 mM/min of GPM initially occurred at about 4.2 mM glucose which attained 10 min after the beginning of the perifusion, and then the insulin release was increased proportionally with increasing glucose levels untill insulin release reached a plateau at the concentration of around 12 mM glucose 50 min after the beginning of the perifusion and the level of insulin release became constant (186±4.6 μU/ml/120 islets/2 min, $N=3$, mean±S.E.M.) at 50 mM glucose (Fig. 3C).

The dose response relationship between the amounts of insulin release and the glucose concentrations under the stimulation with 0.10 mM/min of GPM was sigmoid with a K_m of 7.6 mM glucose, and the insulin release reached a plateau at about 11.1 mM glucose. In the case of stimulation with a rate of gradient level (GPM=0.22 mM/min) a sigmoidal dose response curve was also obtained with the same K_m value as the above (Fig. 4A, Table I).

On the other hand, in the case of glucose stimulation with a rate of gradient level (GPM= 2.80 mM/min) the dose response curve with a K_m value of 20.5 mM (Fig. 4A, B) was obtained.

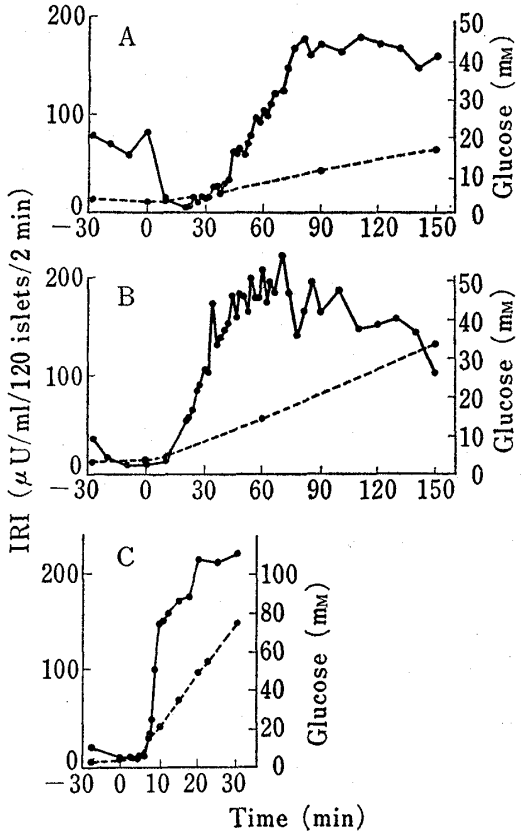

Fig. 3. Dynamics of Insulin Release under a Slow-rise Stimulation

Each point represents the mean insulin released from the perifused rat islets under a slow-rise stimulation by glucose with various rates of linear gradient level, A: 0.10 mM/min (N=3), B: 0.20 mM/min (N=3) and C: 2.80 mM/min (N=3). The broken line shows average levels of glucose in the perifusate collected over each time interval.

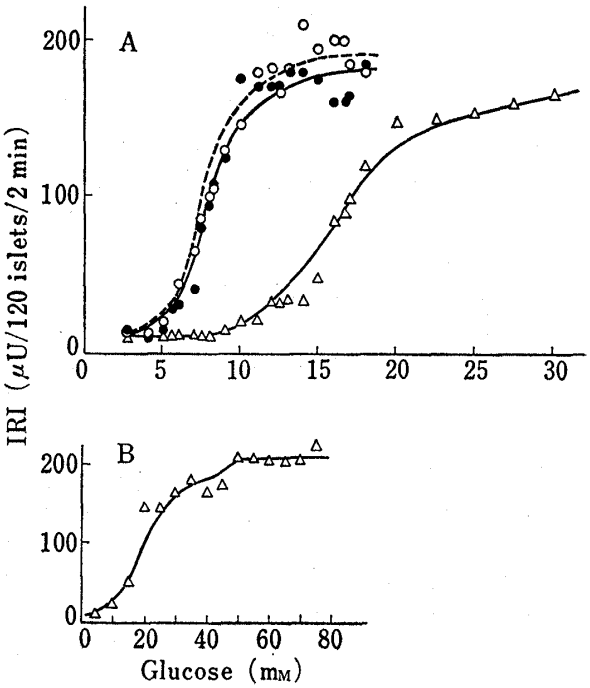

Fig. 4. Dose Response Curves for the Insulin Release under a Slow-wave Stimulation

A: Each point represents the mean values of insulin released from the perifused rat islets under a slow-rise stimulation by glucose with the rates of linear gradient level of 0.10 mM/min (●—● N=3), 0.22 mM/min (○---○ N=3) and 2.80 mM/min (△—△ N=3).

B: The graph indicates the complete dose response curve due to a slow-rise stimulation by glucose with linear gradient level of 2.80 mM/min.

TABLE I. K_m value of Insulin Release from the Rat Islets by Different Stimulation System

Stimulation system		K_m (mM)
Incubation		10.0
Perifusion	square-wave	7.6
	slow-rise GPM=0.10 mM/min	7.6
	0.22 mM/min	7.6
	2.80 mM/min	20.5

Discussion

Insulin release from the perifused rat islets under a square-wave stimulation by glucose ranging from 11.1 to 27.8 mM attained at a plateau followed by constant rates of insulin release within a 30-min period after arrival of the perifusate at the flow cell, while at 5.6 mM glucose

concentration dynamics of insulin release showed only a small peak followed by the decreasing insulin release to the basal level. These results indicate that insulin releasing ability from the B cells stimulated by glucose at the range of 5.6—11.1 mM is much weaker than that by the stimulation with over 11.1 mM glucose. Each dose response curve of the rate of insulin release from islets statically incubated and square-waved glucose-stimulated was represented as a sigmoidal pattern with a K_m value of 10.0 and 7.6 mM glucose respectively. These results suggested that glucose-induced insulin secretory response under these two stimulation systems may be caused by different mechanisms of insulin release each other.

On the other hand, the amount of insulin released under a slow-rise glucose stimulation increased proportionally with the increasing concentration of glucose (4.2—11.1 mM) and then reached a plateau at the concentration of 11.1 mM glucose, which was very similar to a maximal rate of insulin release from the perifused rat islets under a square-wave glucose stimulation. However, the dose response curve for the rate of insulin release by a slow-rise glucose stimulation was represented as a sigmoid curve with a K_m of 7.6 mM glucose and with a tendency to level off at 11.1 mM glucose. Although the stimulation systems were different, insulin secretory response to slowly rising glucose showed the same releasing profile as that due to the square-wave stimulation. Moreover, the slow-rise stimulation by glucose ranging from 4.2 to 11.1 mM produced the maximal change of insulin releasing velocity, and the insulin secretion from the B cell seemed to be most sensitive within the above range of glucose concentration. It was proved that such a sigmoidal profile of insulin release in response to slowly rising glucose may be due to a physiological character of the B cell. Since, a rate of gradient level was raised from 0.10 to 0.22 mM/min, even then, the dose response curve for the rate of insulin release did not alter, it was certain that insulin secretory response dependent to an absolute change of glucose level in the perifusion system.

The stimulation by rapidly rising glucose with 2.80 mM/min of GPM caused an insulin releasing profile similar to that by the square-wave stimulation whereby the glucose level reached a plateau at 27.8 mM within 10 min. Therefore, the rate of insulin release seems to be not dependent on an absolute change of glucose level, and there may be a short delay of insulin release when the islets secrete insulin in response to an unphysiological rapid change of glucose level.

Since, in the postprandial state, it is more likely that glucose absorption from the gut does not occur in an absolute square-wave manner, but rather the blood glucose level gradually increases in a relatively slowly rising manner, insulin releasing profile induced with a slow-rise glucose stimulation would be more physiologic than with a square-wave glucose stimulation.

The sigmoid curve obtained under a solw-rising glucose stimulation in which insulin release initially occurred at 4.2 mM glucose, was very similar to the profile of active potential of the B cell membrane induced with glucose.[6] Moreover, the threshold of glucose for insulin release obtained from the present study was similar to the blood glucose level (4.2 mM) of fasted normal rat,[7] therefore, these results may support that a slow-rising glucose stimulation is similar to physiologic one.

In conclusion, the present study characterizes the procedure for easily available dose response relationship by a slow-rising glucose stimulation and the mechanism of insulin release induced with a slow-rised stimulation by glucose.

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