Sensitized Effect of Prestimulation on Insulin Release from the Perifused Rat Islets with a Slow-Rise Glucose Stimulation

KUNIO KOBAYASHI, YOKO TSUMURA, HISAYO ISE, SHOHEI KAGAWA AND AKIRA MATSUOKA

Department of Clinical Pathology and Clinical Laboratory, Hyogo College of Medicine, 1-1 Mukogawa-cho, Nishinomiya 663, Japan

Synopsis

The sensitized effect of prestimulation with 16.7 mM glucose on insulin release with a slow-rise glucose stimulation from the perifused rat islets of Langerhans was studied, together with the kinetic analysis of insulin release, and the interrelationship between the prestimulation time and the maximal rate of insulin release.

All dose-response relationships which were derived from the dynamics of insulin release from islets prestimulated over various time periods within 60 min, showed sigmoidal profiles. Kinetic analyses were performed with Lineweaver-Burk's and Hill's equations. The 30-min prestimulation significantly reduced Hill's constant ($n$) from $6.2\pm 0.7$ of the control to $3.7\pm 0.6$ ($p<0.05$) and also enhanced the logarithmic equilibrium constant ($\log K$) from $-5.4\pm 0.6$ mM$^{-n}$ to $-3.7\pm 0.6$ mM$^{-n}$ ($p<0.05$). However, the Km value was almost the same as that of the control ($7.3\pm 0.5$ mM).

On the other hand, the 60-min prestimulation remarkably diminished the Km value and the maximal rate of insulin release to $5.3\pm 0.4$ mM ($p<0.05$) and $0.6\pm 0.08$ pU/ml/islet/min ($p<0.005$), respectively. The maximal rate of insulin release linearly increased in proportion to the prestimulation time within 30 min.

In conclusion, these results suggested that there would be some regularity depending on the prestimulation time in the process of transmission of the insulin-releasing signal in the pancreatic B cell and the accumulation of insulin into the provisional pool such as the labile insulin.

A short term prestimulation with glucose enhanced the amount of insulin released with glucose, particularly in the first phase, from the perifused rat islets. It seems that the prestimulation influences the labile pool of insulin in the B cell to induce enhanced insulin release with glucose (Henquin and Lambert, 1974). However, which step through the mechanism of insulin secretion, e.g., the transmission of insulin-releasing signal, glucose metabolism (Coore and Randle, 1964), or the manner of insulin existence in the B cell (Grodsky, 1972) would be sensitized with glucose-prestimulation have not yet been fully explored.

Stimulation with glucose has been well known to depolarize the pancreatic B cell membrane (Dean and Matthews, 1970a), and also induce active potential relating to glucose concentrations (Dean and Matthews, 1970b). Moreover, the dose response curve between the glucose concentration and the intensity of active potential was sigmoid (Dean and Matthews, 1970b) and the intensity of active potential induced with glucose thus seems to be related to the amount of insulin release (Meissner and Atwater, 1976).

We reported in the previous paper that a slow-rise glucose stimulation, however,
would not cause a high depolarization on the B cell membrane (Kobayashi et al., 1977).

The present paper describes the effect of prestimulation with glucose on the dynamics of insulin release from the perfused rat islets under a slow-rise glucose stimulation, those kinetics of insulin release, and inter-relationship between the prestimulation time and the maximal rate of insulin release.

Materials and Methods

Reagents

D-Glucose and theophylline were products of Wako Pure Chemical Industries Ltd., Japan. Bovine serum albumin (fraction V) was purchased from The Armour Laboratories, USA and collagenase from Sigma Chemical Co., USA. Standard pork, 125I-pork insulin, anti-insulin guinea pig serum and anti-r-G-sheep serum were purchased from Dainabot Radioisotope Laboratories Ltd., Japan.

Medium for perifusion

The medium used for all experiments is Krebs-Henseleit bicarbonate buffer (pH 7.35) supplemented with 0.5% bovine serum albumin and 1 mM theophylline, and equilibrated with a mixed gas containing O2 and CO2 (95:5, v/v) and then, D-glucose was added to the medium before use. The partial pressure of oxygen (pO2) in the medium was maintained at 114.3-144.5 mmHg.

Isolation of rat islets

Pancreatic islets of Langerhans were isolated from male Wistar rats made to fast overnight, weighing 120-200 g by the method of Lacy and Kostianovsky (Lacy and Kostianovsky, 1967). Usually, about 120 islets (diameter, 200-250 μm) were obtained from one animal by this method. Fifty islets of comparable size collected from three animals were served for each experiment.

Perifusion system

Fifty islets were perifused in a plastic flow cell maintained at a constant temperature (37°C) and the average flow rate of perfusate was 0.5 ml/min. During the 30-min control period each perfusion system was equilibrated with a medium containing 2.8 mM glucose for 30 min, and then prestimulated with 16.7 mM glucose during the various periods from 0 to 60 min, and again equilibrated with a medium containing 2.8 mM glucose for 30 min. Subsequently, the perifusion was carried out for 120 min under a slow-rise stimulation with glucose which was linearly increased at 0.10 mM/min of the gradient from 2.8 mM glucose. The perfusate was collected by a fraction collector at a 2-min interval.

Measurements

Insulin (Immunoreactive Insulin, IRI) and glucose contents in the effluent fraction were measured by a double antibody radioimmunoassay and Glucose Analyzer (ERA 201, Beckman Instruments, INC., USA) respectively. The partial pressure of oxygen (pO2) in a medium was determined by Blood Gas Analyzer (BMS-series, Denmark Radiometer, Co., Ltd.).

Calculations

All kinetic analyses for the rates of insulin release were performed by Hill's equation (Gutfreund, 1972), which was described in detail in our previous report (Kobayashi et al., 1977) and the statistical comparison was made by Student t test. All calculation were performed with a digital computer (Canola F-20, Canon, INC., Japan).

Results and Discussion

Dynamics of insulin release

Rat islets were stimulated with a slow-rise glucose during the period of the 120-min perifusion after the prestimulation with 16.7 mM glucose was carried out during respective period of 0, 6, 14, 30 and 60 min, as shown in Fig. 1. Either of insulin release reached a plateau at 120 min after commencing perifusion except of a 60-min prestimulation. A slow-rise stimulation followed after the 60-min prestimulation showed a insulin-releasing profile reaching a plateau at 60 min, the amount of insulin being at most about 75% (0.5±0.08 μU/ml/islet/min) of the control.

Dose-response curves for the rate of insulin release

As shown in Fig. 2, the dose-response relationships were sigmoid curves with Km values of 7–9 mM glucose, and with a tendency to a plateau around 13 mM glucose level except the case of the 60-min
prestimulation in which insulin release reached a plateau around 10 mM glucose.

Each Km value was statistically insignificant during the period of prestimulation within 30 min (p<0.10), but the 60-min prestimulation significantly decreased in the Km value to 5-6 mM glucose (p<0.05) (Table 1).

The maximal rate of insulin released by a slow-rise glucose increased in proportion time ranging from 0 to 30 min (Figs. 2 and 3). The decreased effect on insulin release of a long term prestimulation

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Fig. 1. Dynamics of insulin release from the perfused rats islets induced with a slow-rise glucose stimulation after prestimulation with 16.7 mM glucose during various periods. Each curve represents the mean values of three experiments. The broken line shows the mean of each glucose level in the perfusate of 18 experiments.

Fig. 2. Dose response curves for the rates of insulin release under a slow-rise glucose stimulation after prestimulation with 16.7 mM glucose during the various periods. Each point shows the mean value of three experiments and the vertical line represents a S.E.M.

Table 1. Kinetic constants of insulin response to glucose due to the various prestimulation periods

<table>
<thead>
<tr>
<th>Prestimulation period (min)</th>
<th>n</th>
<th>logK (mM^-n)</th>
<th>Km (mM)</th>
<th>Vm (μU/ml/islet/min)</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>6.2±0.7</td>
<td>-5.4±0.6</td>
<td>7.3±0.5</td>
<td>0.8±0.01</td>
<td>6</td>
</tr>
<tr>
<td>6</td>
<td>7.1±0.4*</td>
<td>-6.4±0.3*</td>
<td>8.1±0.3*</td>
<td>1.0±0.02†</td>
<td>3</td>
</tr>
<tr>
<td>14</td>
<td>4.6±0.4*</td>
<td>-4.2±0.4*</td>
<td>8.2±1.0*</td>
<td>1.2±0.03††</td>
<td>3</td>
</tr>
<tr>
<td>30</td>
<td>3.7±0.6**</td>
<td>-3.0±0.6**</td>
<td>6.5±0.5*</td>
<td>1.6±0.28†</td>
<td>3</td>
</tr>
<tr>
<td>60</td>
<td>3.7±0.6**</td>
<td>-2.6±0.5**</td>
<td>5.3±0.4**</td>
<td>0.6±0.08††</td>
<td>3</td>
</tr>
</tbody>
</table>

Each kinetic constant represents the mean values (±S.E.M.) calculated from Hill's equation. * not significant, ** p<0.05, compared with the mean value of the experiments without prestimulation. † p<0.10, †† p<0.05, ††† p<0.005, compared with the mean value of the experiments after the pre-stimulation during shorter period.
tion coincided with the finding that total amounts of insulin release with the second square-wave glucose stimulation following after the first 60-min stimulation by 16.7 mM glucose were significantly reduced to about 60% of the first stimulation, while insulin release in the early phase under the second stimulation increased to about 150% of the control (Table 2). Therefore, it seems likely that a long term prestimulation would cause deficiency of stored insulin proposed by Grodsky (Grodsky, 1972) to diminish insulin release, and that insulin release with a slow-rise glucose stimulation thus would not be from the pool of the early phase insulin, but rather from the pool of the late phase (Curry, 1971).

Table 2. Effect of repeated stimulations on insulin release by glucose

<table>
<thead>
<tr>
<th>Frequency times</th>
<th>IRI (µU/ml/islet/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>min 0-20</td>
</tr>
<tr>
<td>1</td>
<td>0.29±0.01 (100%)</td>
</tr>
<tr>
<td>2</td>
<td>0.43±0.01 (148%)</td>
</tr>
<tr>
<td>3</td>
<td>0.23±0.08 (80%)</td>
</tr>
</tbody>
</table>

The perifusion was carried out under a square-wave stimulation with 2.8 mM glucose and 16.7 mM glucose, and repeated three times. The amounts of insulin released during each period of 16.7 mM glucose stimulation were determined for the first 20 min (min 0-20) and the following 40 min (min 21-60) and expressed as the mean±S.E.M. of three experiments.

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**Fig. 3.** Effects of prestimulation with 16.7 mM glucose during the various periods on the maximal rate of insulin release. The correlation coefficient of the maximal rate of insulin release to the prestimulation period was 0.99. Each point shows the mean value of three experiments. *p<0.05, **p<0.10, ***p<0.005 compared with the mean value of the experiments after the prestimulation during shorter period.

**Fig. 4.** Lineweaver-Burk plots for the rates of insulin released from the perifused rat islets under a slow-rise glucose stimulation after prestimulation with 16.7 mM glucose during the various periods. Each point shows the mean value of three experiments.
Kinetics of insulin release

The kinetic analysis on insulin release with a slow-rise glucose stimulation from the perifused rat islets was performed by Hill's equation, using the results of the dose-response relationship described above.

As shown in Fig. 4, each Lineweaver-Burk plot for the rate of insulin release was not a linear but a parabolic line, suggesting that insulin release may occur at a reaction order higher than the first one.

In order to elucidate the dynamics of insulin release, we now applied Hill's equation represented as follows; \[ \log \frac{v}{V_m-v} = n \log [\text{glucose (mM)}] + \log K. \] The respective values of \( v \) and \( V_m \) represent the rate of insulin release. Hill's constant \( n \) and \( K \) value indicate the cooperativity index between glucoreceptors on the B cell membrane and the affinity of the glucoreceptor to the glucose molecule, respectively.

Each Hill plot for those results was represented as a straight line \((\gamma > 0.98)\) as shown in Fig. 5. Kinetic constants calculated by Hill's equation were demonstrated in Table 1. No significant difference in Hill's constant \( (n) \) among the groups of prestimulation from 0 to 14 min with 16.7 mM glucose was observed, but both of the 30-min and 60-min prestimulations significantly reduced Hill's constant to about 60\% of the control \((n=6.2)\).

As well as Hill's constant, the short term prestimulations did not cause any significant change in the equilibrium constant as compared with the control, while the 30-min and 60-min prestimulations enhanced the equilibrium constant to \(10^{2.4}\) and \(10^{2.8}\) times larger values than that of the control. No difference in \( K_m \) value calculated from Hill's constant was observed between the groups of prestimulation within 30 min, while the 60-min prestimulation significantly diminished the \( K_m \) value as compared with the value in the control \((7.3 \pm 0.5 \text{ mM})\).

Since the \( K_m \) value is calculated by the following equation; \[ K_m = 10^{-\log K/n}, \] the ratio of \( \log K \) to \( n \) does not seem to change each other irrespective of prestimulation time periods within 30 min. Each dose-response curve and its analysis by Hill's equation showed that the threshold of glucose concentration for insulin release and Hill's constant were 5–6 mM and 7.1 in the 6-min prestimulation, and in other cases of longer term prestimulations were 4–5 mM and 3.7–4.6, respectively. A slow-rise glucose stimulation following after the longer prestimulation would become close to a square-wave glucose stimulation since the kinetic constants such as \( n, \log K \) and threshold of glucose for insulin release were similar to each other (Kobayashi et al., 1977).
Moreover, the long term prestimulation by glucose apparently seems to reduce allosteric inhibition between the glucoreceptors through some biophysical changes of intracellular cationic environments by depolarization on the B cell membrane or of accumulation of metabolites and cofactors in the B cell to induce enhanced insulin release at glucose levels lower than 6 mM.

Effect of prestimulation period upon the maximal rate of insulin release

As shown in Fig. 3, the maximal rate of insulin release with a slow-rise glucose stimulation was in proportion to the prestimulation period ranging from 0 to 30 min and was represented as a linear function of time as follows; $V_m = 0.028t + 0.82$ ($r = 0.99$)

Increase in the maximal rate of insulin release sensitized by prestimulation would be caused by the enhancement of glucose metabolism and of the dissociation from the bound Ca++ to free Ca++ in cytosol, the latter of which is known to stimulate the microtubulus-microfilamentous system to elicit augmented insulin release from the B cell (Lacy et al., 1970; Malaisse and Malaisse-Lagae, 1970), and by the increased accumulation of insulin into the provisional pool such as the labile insulin suggested by Grodsky (1972). Therefore, the above intracellular biological variations in islets influencing insulin release would be regulated by the prestimulation period.

In conclusion, it has been suggested that the prestimulation within 30 min may enhance insulin release with a slow-rise glucose stimulation in the allosteric inhibition between glucoreceptors and increase in labile pool of insulin, and on the other hand, the longer one may diminish the maximal rate of insulin release by deficiency of stored insulin, whereas it lowered the threshold of glucose concentration for insulin release.

In order to assess further the mechanism of increased insulin release depending on the prestimulation time and the significance of kinetic constants, there would be needed studies on the metabolism of glucose and the binding manner between glucose and glucoreceptors.

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References