Characteristics of the Biotin Enhancement of Glucose-induced Insulin Release in Pancreatic Islets of the Rat

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Perifused isolated rat islets were used to show that biotin plus 16.5 mM glucose evoked more insulin secretion than 16.5 mM glucose alone. Whether or not this reinforcement of glucose-induced insulin secretion by biotin is unique was studied by using perifused islets stimulated with 16.5 mM glucose plus 100 μM of one of various components of the vitamin B group. No effect of any of these vitamins was found on glucose-induced insulin secretion. These results indicate that biotin is unique among the members of the vitamin B group in enhancing glucose-induced insulin secretion.

Static incubation experiments showed that biotin did not potentiate insulin release when the islets were incubated with an experimental solution containing either no or 2.8 mM glucose. The addition of biotin to 27.7 mM glucose, which is the maximal concentration for stimulating insulin release, did not significantly enhance the effect of the glucose on insulin release (although it did at 16.5 mM glucose). These findings indicate that biotin, by itself, does not stimulate insulin secretion, and does not enhance glucose-induced insulin secretion beyond the ability of glucose itself to stimulate insulin secretion.

Key words: biotin; insulin release; pancreatic islet; vitamin B group

The biochemical role of biotin in CO₂ fixation is well established, and many metabolic functions have been ascribed to this vitamin. Several early reports have indicated that a biotin deficiency may induce an impairment of glucose utilization. Several years ago, we reported that, in the early stages of biotin deficiency in rats, the plasma insulin level during an oral glucose tolerance test was lower than that seen in the control animals, even though the plasma glucose levels were almost the same in the two groups. We later showed that this reduced insulin secretion in biotin-deficient rats was improved by the simultaneous administration of biotin (1 mg/kg of body weight) with a glucose solution. In our recent study using an isolated perfused pancreas, we found that the insulin-releasing response to 20 mM glucose in biotin-deficient rats was approximately 22% of that seen in the control rats, again indicating that insulin release from the pancreas is disturbed in biotin-deficient rats. Furthermore, a reinforcement of the glucose-induced insulin secretion by biotin was found not only in biotin-deficient rats, but also in normal rats. These biotin-induced increases in glucose-induced insulin release were evident within the first few minutes of the infusion. Moreover, the relationship between the biotin concentration in a stimulus solution containing 20 mM glucose and the insulin response to glucose + biotin (expressed relative to the response to glucose alone) showed a highly significant correlation. These observations led us to believe that biotin might exert an important modulating influence on the mechanism underlying glucose-induced insulin secretion. However, it is not known whether biotin itself has the ability to induce insulin secretion, or whether other vitamins can enhance glucose-induced insulin secretion in this experimental system. In the present study, we investigated the characteristics of the biotin-induced enhancement of glucose-induced insulin secretion by using perfusion of, and static incubation with pancreatic islets isolated from rats.

Materials and Methods

Isolation of the islets. In each experiment, male Wistar rats, 12 weeks old, obtained from Funabashi Farm (Shizuoka, Japan) were used to provide isolated pancreatic islets. The pancreatic islets were isolated by a collagenase digestion technique and separated by centrifuging the digested pancreas in a discontinuous density gradient with a dextran solu-

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Abbreviations: B₆, thiamine-2 phosphate; B₆, riboflavin; B₉, pyridoxine; B₁₂, cyanocobalamin; NA, nicotinic acid; PA, pantothenic acid; FA, folic acid; KRBB, Krebs-Ringer bicarbonate buffer; IRI, immunoreactive insulin
tion (Dextran T70; Pharmacia Biotech, Uppsala, Sweden). The isolated islets were cultured for 24 h in an RPMI 1640 medium containing 10% fetal bovine serum, 2 mM glutamine, 100 U/ml of penicillin, and 100 μg/ml of streptomycin before the study on insulin release was begun.

Pancreatic islet perfusion. Thirty to thirty-five isolated islets were pooled into a plastic flow-through perfusion minichamber. The baseline perfusate consisted of a Krebs-Ringer bicarbonate buffer (KRBB) containing 0.2% BSA, 5.6 mM glucose, and 5 mM each of pyruvate, fumarate and glutamate (pH 7.4). The perfusate was continually oxygenated in an atmosphere of 95% O₂ and 5% CO₂. After a 90-min preperfusion period, the islets were perfused for 10 min with the baseline perfusate. This was followed by a 30-min test with 16.5 mM glucose, a 60-min interval with the baseline perfusate, and finally by a 30-min test with 16.5 mM glucose + one of a number of concentrations of biotin (0, 10 μM, 100 μM, or 1 mM). In the study on the uniqueness of the biotin effect, 100 μM of one of a number of vitamins (thiamine-2 phosphate (B₁), riboflavin (B₂), pyridoxine (B₆), cyanocobalamine (B₁₂), nicotinic acid (NA), pantothenic acid (PA), or folic acid (FA)) instead of biotin was added to a 16.5 mM glucose solution to stimulate insulin secretion. The flow rate was maintained at 1.0 ml/min. Samples were stored at −20°C until being assayed for immunoreactive insulin (IRI).

Static incubation experiment with the islets. All the size-matched islets available were preincubated with KRBB containing 0.2% BSA (equilibrated with an atmosphere of 95% O₂ and 5% CO₂ at pH 7.4) for 60 min. After a preincubation, 10 islets/tube were incubated for 30 min with one of a number of concentrations of glucose (0, 2.8, 5.6, 16.5, or 27.7 mM) or with one of the same concentrations of glucose plus 100 μM biotin. Immediately thereafter, the supernatant was collected and stored at −20°C until being assayed for IRI.

Table 1. Insulin Secretion during the Perfusion of Isolated Islets with 16.5 mM Glucose and 16.5 mM Glucose Plus a Given Concentration of Biotin

<table>
<thead>
<tr>
<th>Biotin concentration</th>
<th>Immunoreactive insulin (pmol/l)</th>
<th>Relative insulin release (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>16.5 mM glucose</td>
<td>16.5 mM glucose + biotin</td>
</tr>
<tr>
<td>0 μM</td>
<td>229 ± 24</td>
<td>224 ± 21</td>
</tr>
<tr>
<td>10 μM</td>
<td>221 ± 25</td>
<td>283 ± 29</td>
</tr>
<tr>
<td>100 μM</td>
<td>229 ± 23</td>
<td>345 ± 37</td>
</tr>
<tr>
<td>1 mM</td>
<td>251 ± 29</td>
<td>363 ± 48</td>
</tr>
</tbody>
</table>

Each value is the mean ± SEM, and represents the mean integrated insulin-releasing response. Relative insulin release was calculated by dividing the integrated insulin secretory response to 16.5 mM glucose plus a given concentration of biotin by that to 16.5 mM glucose, and expressing the result as a percentage. The integrated insulin secretory response for each stimulus was calculated by subtracting the baseline level (5.6 mM glucose) from the absolute insulin level (n = 6). a: significant against the value in 0 mM biotin (p < 0.01). b: significant against the value in 10 μM biotin (p < 0.05).

Determination of the insulin level. IRI in the pancreatic islet effluent was determined by an Insulin RIA kit (Shionogi and Co., Osaka) which employs the double-antibody radioimmunoassay method.

Statistical analysis. Each data value is expressed as the mean ± SEM. The statistical significance of differences was assessed by using Student’s t-test or one-way ANOVA with Turkey’s multiple-comparison test, p < 0.05 being accepted as the level of significance.

Results and Discussion

Relationship between glucose-induced insulin secretion and biotin concentration

In the first experiment, the effect of repeated glucose stimulation on insulin secretion was investigated by using isolated pancreatic islet perfusion (Fig. 1 and Table 1). The mean integrated insulin response to the first stimulus with 16.5 mM glucose was the

Fig. 1. Insulin Secretory Response Evoked by Isolated Perfused Islets of Rats.

Insulin secretory responses were evoked by 16.5 mM glucose (from t = 10 to t = 40 min) and 16.5 mM glucose plus 100 μM biotin (from t = 100 to t = 130 min). Each point represents the mean ± SEM (n = 6).
same as that to the second stimulus with 16.5 mM glucose (229 ± 24 pmol/l and 224 ± 21 pmol/l, respectively). In some previous studies on pancreatic islet perfusion, a second glucose pulse gave rise to a greater insulin response than the first and third glucose pulses, possibly due to a priming effect of the first glucose pulse, although this was not certain. However, in the present study, the magnitude of the insulin response to the second glucose stimulus was not significantly different from that evoked by the first (Table 1). The discrepancy between our results and previous findings may have resulted from differences in the experimental conditions. In our study, an interval of 60 min was allowed between the first stimulus and the second for a washout perfusion with the baseline perfusate; this was three times as long as that allowed in the previous studies. In addition, basal insulin secretion just before the second glucose stimulation was not significantly different from that just before the first stimulus (data not shown, but the levels can be seen in Fig. 1). It thus seems likely that the prolonged effect of the first glucose stimulus weakened over the course of the long basal perfusion, and that, in the present study, the state of the islets (before the glucose stimulus) was not different between the first and second stimuli.

The responses to glucose and to the glucose-plus-biotin combinations are also shown in Fig. 1 and Table 1. The insulin release seen in response to 16.5 mM glucose plus 100 μM biotin was increased to 150 ± 4% of the response to 16.5 mM glucose without biotin. Moreover, this enhancing effect of biotin was evident within a few minutes of the start of the perfusion with a biotin-containing glucose solution. This increase persisted until the end of the stimulation with 16.5 mM glucose plus 100 μM biotin (Fig. 1). Furthermore, the insulin release seen in response to 16.5 mM glucose plus biotin (10 μM or 1 mM) was, at each biotin concentration, greater than the response to 16.5 mM glucose alone (128 ± 6% or 144 ± 3%, respectively, of the response to 16.5 mM glucose). In addition, the relative insulin release was significantly less with 10 μM biotin than with 100 μM or 1 mM biotin (p < 0.05 in each case). However, the relative insulin release seen with 100 μM biotin was not significantly different from that seen with 1 mM biotin, although, in our previous study, the relationship between the biotin concentration (1 μM–1 mM) and its effect on the insulin-releasing response to glucose showed a highly significant correlation. This discrepancy may be due to our use of a whole pancreas or only of the isolated islets cultured in an RPMI 1640 medium for 24 h (the present study). Indeed, it has been reported that there was a difference in insulin secretion between fresh islets and cultured islets in a high-glucose medium. With the above-mentioned exception, the present results agree well with our previous findings that were obtained by perfusion of the isolated pancreas.

**Uniqueness of the enhancing effect of biotin on glucose-induced insulin secretion**

It has been reported that the vitamin status influenced glucose metabolism. In particular, it is well known that B1, a member of the vitamin B complex, plays an important role in glucose metabolism as a cofactor of the pyruvate dehydrogenase complex (which catalyzes pyruvate to acetyl-CoA). Of the other members, B3, and PA as a precursor of CoA are also related to the pyruvate dehydrogenase complex. Moreover, CoA is involved in the conversion of α-ketoglutarate to succinyl CoA in the Krebs cycle. In addition, some other vitamins could also influence insulin secretion from the pancreatic B cell: for example, (1) vitamin D-deficient animals could impair insulin secretion, or vitamin D could enhance insulin secretion, and (2) vitamin A and vitamin C may influence insulin secretion. However, it has not been established whether each vitamin in the vitamin B complex can enhance glucose-induced insulin secretion, as biotin does. The effect of vitamins other than biotin on glucose-induced insulin secretion was studied by using a perfusion containing 16.5 mM glucose plus 100 μM of one of seven vitamins from the vitamin B group (some of them related to glucose metabolism as already described) (Table 2). In this study, these vitamins did not have any effect on 16.5 mM glucose-stimulated insulin secretion like that seen in the presence of 100 μM biotin. The relative insulin release was significantly weaker than that in the presence of biotin (relative insulin release: 106 ± 6%, 91 ± 4%, 95 ± 10%, 96 ± 11%, 93 ± 8%, 97 ± 3%, and 104 ± 14% in the presence of 100 μM of B1, B3, B12, NA, PA, or FA, respectively; p < 0.01 for each vitamin versus the relative insulin release seen in the presence of 100 μM biotin). These data indicate that, among the members of the vitamin B group examined, only biotin was proved to be able to enhance glucose-induced insulin secretion.

**Static incubation experiment with the islets**

Glucose, the major insulin secretagogue, stimulates insulin release following its metabolism via glycolysis and the Krebs cycle. The maximal effective concentration of glucose is 27.7 mM for stimulating insulin release, and a glucose concentration under 3.3 mM did not potentiate the insulin release evoked by a depolarizing concentration of K+. In the present static incubation experiment, 100 μM biotin enhanced the 16.5 mM glucose-induced insulin release to the same degree as that seen in the isolated pancreatic islet perfusion experiment (compare Table 3 with Table 1). However, the level of insulin release seen when the incubation solution containing
Table 2. Insulin Secretion during the Perifusion of Isolated Islets with 16.5 mM Glucose and 16.5 mM Glucose Plus 100 μM of a Given Vitamin

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>n</th>
<th>Immunoreactive insulin (pmol/l)</th>
<th>Relative insulin release (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>16.5 mM glucose</td>
<td>16.5 mM glucose + 100 μM vitamin</td>
</tr>
<tr>
<td>B₁</td>
<td>6</td>
<td>286 ± 19</td>
<td>293 ± 11</td>
</tr>
<tr>
<td>B₂</td>
<td>6</td>
<td>313 ± 21</td>
<td>282 ± 15</td>
</tr>
<tr>
<td>B₆</td>
<td>7</td>
<td>311 ± 30</td>
<td>297 ± 31</td>
</tr>
<tr>
<td>B₁₂</td>
<td>7</td>
<td>319 ± 47</td>
<td>306 ± 38</td>
</tr>
<tr>
<td>NA</td>
<td>9</td>
<td>312 ± 32</td>
<td>290 ± 26</td>
</tr>
<tr>
<td>PA</td>
<td>6</td>
<td>313 ± 25</td>
<td>301 ± 20</td>
</tr>
<tr>
<td>FA</td>
<td>6</td>
<td>319 ± 46</td>
<td>332 ± 39</td>
</tr>
<tr>
<td>Biotin</td>
<td>6</td>
<td>319 ± 16</td>
<td>498 ± 29</td>
</tr>
</tbody>
</table>

Each value is the mean ± SEM, and represents the mean integrated insulin-releasing response. The integrated insulin secretory response and relative insulin release were calculated as described in the legend to Table 1 (n = 6).

Table 3. Insulin Release during Static Incubation of Islets with a Given Concentration of Glucose with or without 100 μM Biotin

<table>
<thead>
<tr>
<th>Glucose concentration (mM)</th>
<th>Immunoreactive insulin (pmol/l/10 min/10 islets)</th>
<th>Relative insulin release (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No biotin</td>
<td>n</td>
</tr>
<tr>
<td>0.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16.5</td>
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<tr>
<td>27.7</td>
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</table>

Each value is the mean ± SEM. Relative insulin release was calculated by dividing the insulin release seen with a given concentration of glucose plus 100 μM biotin by that seen with the same concentration of glucose.

100 μM biotin without glucose was almost the same as that seen during the incubation with a basal solution (286 ± 79 pmol/l/10 min/10 islets and 280 ± 34 pmol/l/10 min/10 islets, respectively; Table 3). The level of insulin release induced by the 2.8 mM glucose plus 100 μM biotin combination was 366 ± 55 pmol/l/10 min/10 islets, and this was not significantly different from that seen with 2.8 mM glucose alone (Table 3). It is well known that secretagogues do not need a substantial amount of nutrient to induce insulin secretion. For example, 2-ketoisocaproate and methyl succinate are themselves mitochondrial fuel, while arginine, which is a well-known secretagogue for insulin secretion, needs only a small amount of a nutrient such as 2.8 mM glucose. To judge from these reports, the data obtained in this study indicate that biotin, by itself, did not stimulate insulin secretion by acting as a secretagogue. Moreover, the addition of 100 μM biotin to 27.7 mM glucose, which is the maximal concentration for stimulating insulin release, induced a release of insulin by 1466 ± 234 pmol/l/10 min/10 islets, a value not significantly greater than the insulin release seen with 27.7 mM glucose alone (Table 3). This finding indicates that biotin did not enhance glucose-induced insulin secretion beyond the ability of glucose itself at this concentration to stimulate insulin secretion. By contrast, when biotin was used in combination with glucose below 27.7 mM, the insulin release increased progressively (Table 3).

In conclusion, our present results indicate that biotin enhanced glucose-induced insulin release from the pancreatic islets of the rat, but did not act as an insulin secretagogue by itself. This effect on glucose-induced insulin release is unique to biotin among the members of the vitamin B group examined.

References
6) Furukawa, Y., Satoh, H., Sakamoto, A., Koizumi,


