Reversal of Glucose Insensitivity of Pancreatic B-Cells Due to Prolonged Exposure to High Glucose in Culture: Effect of Nicotinamide on Pancreatic B-Cells

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Ohgawara, H., Kawamura, M., Honda, M., Karibe, S., Iwasaki, N., Tasaka, Y. and Omori, Y. Reversal of Glucose Insensitivity of Pancreatic B-Cells Due to Prolonged Exposure to High Glucose in Culture: Effect of Nicotinamide on Pancreatic B-Cells. Tohoku J. Exp. Med., 1993, 169 (2), 159-166 —— Prolonged in vitro exposure to high glucose has been shown to induce a decrease in pancreatic B-cell sensitivity to glucose stimulation. This experiment was designed to study whether nicotinamide affects the B-cells’ response to regulatory stimuli of glucose after prolonged culture with high glucose. Neonatal pig pancreatic islet-like cell clusters (ICCs) formed from single cells were embedded in agarose-gel. Some of them were maintained in RPMI 1640 containing a high glucose concentration (16.7 mM) and 10% fetal bovine serum (FBS) with or without nicotinamide. Then, the embedded-ICCs were stimulated by glucose at concentrations of 5.5 mM and 16.7 mM. After the prolonged culture with high glucose, the ICCs showed no response to acute glucose stimulation. There was a significant increase in glucose-mediated insulin secretion when the ICCs were maintained with the medium containing nicotinamide. We conclude that nicotinamide could protect B-cell desensitization to glucose after prolonged exposure to high glucose. —— desensitization to glucose; pancreatic islet; ICCs; agarose; embedded-culture

It has been reported that when pancreatic B-cells are chronically exposed to high glucose, subsequent insulin release in response to glucose is impaired (Cerasi et al. 1972; Bolaffi et al. 1986; Hoenig et al. 1986; Purrello et al. 1989). Unger and Grundy reported that a long-standing severe hyperglycemia may irreversibly damage B-cells (1985). This has been demonstrated both in vitro and in vivo.

Nagamatsu et al. have observed that cultured rat islets chronically exposed to high glucose become desensitized to subsequent glucose-stimulated insulin secretion (1987). Furthermore, Weir et al. demonstrated that the B-cell ability to suppress insulin secretion when plasma glucose concentrations are low was impaired in normal subjects in whom hyperglycemia was maintained using the clamp
technique (1981). Also, the altered insulin secretion of diabetic patients (non-insulin dependent diabetes) was improved by correcting hyperglycemia with diet, hypoglycemic agents, or insulin (Savage et al. 1979; Kosaka et al. 1980).

Recently, Yonemura et al. (1984) showed that a poly (ADP-ribose) synthetase inhibitor, such as nicotinamide, can prevent diabetes in partially depancreatized rats.

In this study, we aimed to protect the B-cell desensitization to glucose by using nicotinamide in the culture medium during the incubation period.

We observed glucose-stimulated insulin secretion after a prolonged exposure to high glucose with or without nicotinamide.

**MATERIALS AND METHODS**

*Preparation of islet cells.* Pancreatic islet-like cell clusters (ICCs) were prepared from neonatal pig pancreases by modifying the method of Archer (1983), as previously reported from our laboratory (Ohgawara et al. 1991).

*Embedded-culture of ICCs.* Agarose was commercially prepared Agarose-LGT (polymerized at low temperature: Nakalai tesque Inc., Tokyo). A 2.0% (W/V) solution of agarose was sterilized in an auto-sterilizer for 30 min. Then, the agarose solution and a 2X concentration of RPMI 1640 containing 20% FBS with or without 1.0 or 10 mM nicotinamide were mixed in equal amounts. Approximately 5,000 ICCs were added to the above mixture to give a final density of 200-250 ICCs per ml of the agarose mixture. One ml of the mixture was placed in a multiwell plate (24-wells; Corning Glass Works, NY, USA), and then brought to 30°C to allow polymerization of the agarose. The incubation was continued at 37°C under an atmosphere of 5% CO$_2$ and air. The cultures were fed with RPMI 1640 containing 16.7 mM D-glucose, 10% FBS and with or without 10 mM nicotinamide. From the following day, the medium was replaced every 3 days with RPMI 1640 supplemented with or without nicotinamide.

*Glucose-stimulated insulin release.* Insulin secretion to glucose was observed on the 1st, 7th and 21st culture days. The culture medium was changed 4 hr before the short-term glucose-stimulated insulin secretion. The tissue culture medium was replaced with 1.0 ml of RPMI 1640 containing 10% FBS and 3.3 mM D-glucose. Preincubation was performed to establish a stable baseline for insulin secretion. The preincubation medium was then discarded and replaced with 1.0 ml of a medium containing 5.5 mM or 16.7 mM D-glucose. The incubation media were collected at the end of the 5-hr culture period and centrifuged, and the supernatants were stored at -20°C for insulin assay.

**RESULTS**

Chronic stimulation of the ICCs with culture media containing high glucose, with or without nicotinamide, was characterized by the insulin output in response to glucose. The ICCs embedded in agarose-gel were maintained in RPMI 1640 containing 16.7 mM D-glucose and 10% FBS with or without nicotinamide (1.0 or 10 mM) for several days. They were then stimulated by glucose. As shown in Fig. 1, when the culture medium contained 10 mM nicotinamide the insulin output in response to glucose (16.7 mM) was much higher than that in ICCs which had been maintained in the culture medium containing 16.7 mM D-glucose alone, except on day 1 in response to 5.5 mM D-glucose.
Fig. 1. Insulin output in response to different concentration of glucose (5.5 mM or 16.7 mM) by pancreatic islet cell-clusters (ICCs) cultured in RPMI 1640 plus 10% fetal bovine serum under the three different culture conditions: 1) glucose (16.7 mM) alone; 2) glucose and a low concentration (1.0 mM) of nicotinamide; and 3) glucose and a high concentration (10 mM) of nicotinamide.

Fig. 2. Basal and glucose-stimulated insulin secretion from embedded-ICCs in agarose with or without nicotinamide. The ICCs-cultures were incubated for 5 hr in 1.0 ml of RPMI containing D-glucose (16.7 mM) alone; D-glucose and 1.0 mM nicotinamide; or D-glucose and 10 mM nicotinamide on days 1, 7 and 21 of culture. The glucose-stimulated insulin secretion from the ICCs was calculated as follows:

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\text{IRI secretion} \times 100 = \frac{\text{IRI response to 5.5 or 16.7 mM D-glucose on day 1, 7 or 21 of culture}}{\text{IRI response to 5.5 mM D-glucose on day 1 of culture}}
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Fig. 3. Morphology of the ICCs. The embedded ICCs in agarose and nicotinamide observed with a light microscope after enzymatic staining. (100×) The ICCs are well preserved in the agarose. INS, B-cell; GLU, A-cell; SOM, D-cell; PP, pp-cell.
When comparing the data in Fig. 2, the increase in the glucose concentration from 5.5 to 16.7 mM in the incubation medium did not significantly affect the glucose-stimulated state when estimated after prolonged culture with high glucose alone. On the 1st day, insulin secretion in response to glucose was 100% of the control in 5.5 mM D-glucose-stimulation and 85% of the control in 16.7 mM D-glucose after a prolonged culture with high glucose (16.7 mM) alone. On day 7, it was 39% of the control in the 5.5 mM D-glucose and 47% of the control in the 16.7 mM D-glucose. On day 21, it was 20% of the control in the low glucose and 24% of the control in the high glucose. The basal insulin secretion to glucose was decreased during the culture periods and the ICCs showed a very poor increase in insulin secretion in response to the glucose challenge.

In the presence of nicotinamide, the insulin response to glucose was much higher than that of the ICCs which had been maintained with glucose alone. The ICCs exposed during the seven days of culture to 16.7 mM D-glucose with 1.0 or 10 mM nicotinamide, showed a significantly higher glucose-stimulated insulin release than that of the ICCs which had been maintained in 16.7 mM D-glucose alone ($p < 0.05$).

On day 7 of culture, the basal insulin secretion to 5.5 mM D-glucose showed a similar insulin release to the glucose on the 1st day, in the presence of 10 mM nicotinamide (100% of control in response to the low glucose on day 1 vs. 98% of control on day 7) but not with 1.0 mM nicotinamide.

The ICCs which were embedded in agarose-gel were observed with a light microscope after enzymatic staining. The ICCs were found to be intact on day 21 of culture (Fig. 3).

**Discussion**

The present study shows that nicotinamide reduced glucotoxicity after chronic exposure of normal pancreatic B-cells to a high glucose concentration (16.7 mM D-glucose) in vitro. During a prolonged culture in the presence of 16.7 mM D-glucose, ICCs showed a very poor increase in insulin release in response to an acute glucose challenge, on the 1st day of culture, but not in the presence of nicotinamide.

Yarimizu et al. demonstrated "desensitization" in a perifusion system using cultured islets. When the islets had been previously cultured in high glucose for few days, the amount of insulin release in response to high glucose showed a marked decrease in both the first and the second phases. They also observed that reversibility of impaired insulin secretion by high glucose occurred when the desensitized islets were subsequently cultured in low glucose (1991).

Giroix et al. (1989) reported that glucose insensitivity in 1-day cultured islets was primarily caused by a direct deleterious effect of high glucose concentration on the B-cells. Cerasi et al. (1972) reported that the glucose insensitivity is due to a decrease in the sensitivity of the glucose receptors of the pancreatic B-cells.
which transmits the glucose signal for insulin release.

Dunbar et al. (1989) suggested that the glucose desensitization of the B-cells might be due to alteration of the calcium-dependent release mechanism. And Okamoto et al. (1992) also reported that the inability of glucose to provoke a $[\text{Ca}^{2+}]_i$ rise, which is observed in the B-cells exposed to high glucose may be responsible for the selective impairment of the glucose-induced insulin secretion.

In our experiment, nicotinamide protected ICCs from desensitization in response to the acute glucose stimulation after prolonged culture in the presence of 16.7 mM D-glucose.

It was reported by Okamoto (1991) that nicotinamide, an inhibitor of islet nuclear poly (ADP-ribose) synthetase, reversed the reduction in NAD level and also inhibition of proinsulin synthesis. It was also found that in rat insulinoma cells cultured in the presence of streptozotocin, nicotinamide increased DNA single-strand breaks in the cells indicative of the inhibition of the poly (ADP-ribose) synthetase, but not in the presence of alloxan (Yamamoto et al. 1981; Uchigata et al. 1982; Willson et al. 1984). Furthermore, nicotinamide prevented or slowed the manifestation of diabetes in non-obese diabetic (NOD) mice (Yamada et al. 1982; Nakajima et al. 1985).

Masiello et al. (1985) observed a protective effect of 3-aminobenzamide (a potent inhibitor of poly (ADP-ribose) synthetase) against streptozotocin-induced diabetes, and that the protective effect was similar to that exerted by nicotinamide. Therefore, they strongly supported the idea that the effect may be a major consequence of the activation of DNA repair mechanism in islet cells.

Recently, Varsányi-Nagy et al. (1992) reported that nicotinamide preserved islet structure and stimulated insulin release and synthesis in an in vitro islet culture system. They also observed that the effect was amplified by addition of low-dose IL-1B.

More recently, Takasawa et al. (1993) reported that cyclic (ADP ribose) is a mediator of calcium release from islet microsomes and might be generated in the islets by glucose stimulation, serving as a second messenger for calcium mobilization in the endoplasmic reticulum.

In our experiment, the presence of nicotinamide may be a major consequence of the activator of NAD+ mechanisms in islet cells.

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