Dependence of Cholecystokinin-8-Stimulated Insulin Release on High Glucose Levels Is Evidenced by Pseudo-α-D-Glucose in Rat Pancreas and Islets

Ikuko Kimura¹, Noboru Nakashima¹, Tokie Komori¹, Yukihiro Kameda² and Masayasu Kimura¹

¹Department of Chemical Pharmacology, Faculty of Pharmaceutical Sciences, Toyama Medical and Pharmaceutical University, Toyama 930–01, Japan
²Department of Biochemistry, School of Pharmacy, Hokuriku University, Kanazawa 920–11, Japan

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ABSTRACT—The glucose-dependency of cholecystokinin-8 (CCK-8)-stimulated insulin release was investigated at high (11.1 and 16.7 mM) glucose concentrations in rat pancreas perfusion and islet perifusion using glucose analogues, pseudo-α-D-glucose and 2-deoxy-D-glucose. In perfused pancreas, both glucose analogues (22.4 mM) inhibited high glucose (16.7 mM)-induced insulin release, but not normal glucose (5.6 mM)-induced insulin release, with or without CCK-8 (1 nM). In perifused islets, the same level of either of the glucose analogues inhibited high glucose (11.1 mM)-induced insulin release, with or without CCK-8 (100 nM). These results demonstrate that CCK-8-stimulated insulin release only at high glucose level is glucose-dependent.

Keywords: Cholecystokinin-8 (CCK-8), Insulin release, Pancreas (rat perfused), Islet (rat perifused), Pseudo-α-D-glucose

Cholecystokinin (CCK) modulates the secretory activity of pancreatic B cells (1, 2), which has been supported by specific binding studies for CCK in pancreatic islets (3, 4). CCK-8 releases insulin glucose-dependently either from perfused pancreas or isolated rat islets (4, 5), because CCK-8-induced stimulation of insulin release requires the presence of an intermediate stimulatory glucose level of more than 5 mM (6). There are two major theories about how glucose stimulates insulin release from the pancreatic islets: one is that glucose itself modifies a plasma membrane-bound glucoreceptor (7), and the other is that a metabolite or cofactor for insulin release is generated by the metabolism of glucose in pancreatic B cells (8). Glucokinase (α-glucose 6-phosphotransferase) plays an important role in controlling glucose phosphorylation and metabolism in pancreatic islets (9). The functions of pancreatic islets are mainly explained by glucokinase (8, 10). The hypothesis that glucokinase is a glucose-sensor, therefore, has been widely reviewed and accepted in stimulus-secretion coupling of pancreatic B cells (9–11). A synthetic analogue of glucose anomer, pseudo-α-D-glucose, is effective in inhibiting the activity of islet glucokinase and glucose-induced insulin release (10). The aim of the present study is to investigate the glucose-dependency of CCK-8-stimulated insulin release using pseudo-α-D-glucose, especially at high glucose levels.

MATERIALS AND METHODS

Male Wistar rats (200–300 g) were used. Rats were anesthetised with pentobarbital (Nembutal, 50 mg/kg, i.p.; Abbott Lab., North Chicago, IL, USA), and then the pancreas was isolated and perfused as reported previously (12). Perfusion was carried out through the celiac artery with a basal medium of Krebs-Ringer bicarbonate buffer solution (pH 7.4) containing 0.5016 bovine serum albumin (Fraction V; Sigma, St. Louis, MO, USA), 2% dextran (T-70; Pharmacia, Uppsala, Sweden) and 2.8 mM D-glucose (13), saturated with a gas mixture of 95% O₂ and 5% CO₂ at 37 °C.

The islets were isolated with collagenase (Type IV; Worthington Biochemical Corp., Freehold, NJ, USA)-digestion (14, 15) and perifused with a slight modification of the procedure (16, 17). Usually 30–40 islets were collected from a rat. The islets were placed in a finntip (a volume of 150 μl; Labsystems Corp., Helsinki, Finland)-chamber coated with collagen (Type I; Koken, Tokyo)-acetic solution, and they were perifused at a flow rate of 1
ml/min with Krebs-Ringer bicarbonate buffer solution (pH 7.4) containing 0.5% bovine serum albumin and 2.8 mM glucose, which was saturated with a gas mixture of 95% O₂ and 5% CO₂ at 37°C. The islets were first perfused at a low flow rate (10 ml/hr) for 60 min in order to make them adhere to the chamber and secondly, at a basal rate (1 ml/min) for 60 min to obtain stable basal secretory rates. Then the 10-min stimulation by glucose alone and that by glucose plus CCK-8, with or without glucose analogues, were carried out according to the protocol.

Insulin, from the perfused pancreas through the portal vein and from perfused islets, was assayed by radioimmunoassay as reported previously (18). The results were statistically analyzed by Student’s unpaired t-test. Pseudo-α-D-glucose was a gift from Dr. I. Kitagawa (Department of Pharmacognogy, Faculty of Pharmaceutical Sciences, Osaka University, Suita) and was prepared by Dr. Y. Kameda, one of the present authors. CCK-8 (a sulfated form) was purchased from the Peptide Institute, Inc. (Osaka) and 2-deoxy-D-glucose, from Nacalai Tesque (Kyoto).

RESULTS

Glucose analogues do not inhibit CCK-8-stimulated insulin release at normal glucose level in isolated perfused rat pancreas

A 30-min stimulation with CCK-8 potentiated two distinctively different phases of normal glucose level (5.6 mM)-induced increase of immunoreactive insulin (IRI) level in perfused rat pancreas. There was an early and acute rise (the first phase) in IRI release that fell within 6 min, followed by a late and slow release (the second phase). When CCK-8 was present, the IRI level was significantly higher than that obtained with glucose-stimulation alone. Furthermore, the potentiating effect at the first phase was more remarkable than that at the second phase. All the experiments thereafter, were done with 10-min stimulation. The potentiation by CCK-8 of insulin release at 5.6 mM glucose was concentration-dependent (from 0.25 to 2.0 nM). The EC₅₀ (95% confidence limits) was 0.47 (0.42–0.53) nM, and the submaximal response was obtained at 1.0 nM. CCK-8 (1 nM) with or without 2.8 mM glucose did not influence the basal rate of IRI release (data not shown).

The glucokinase inhibitor pseudo-α-D-glucose or the metabolic inhibitor 2-deoxy-D-glucose (2DG), either at 22.4 mM, inhibited the glucose-concentration-dependent release of IRI (Fig. 1A). Glucose (16.7 mM)-induced IRI release was inhibited significantly (P<0.01) by 11.2 and 22.4 mM glucose analogues. The inhibition by 2DG was stronger than that by pseudo-α-D-glucose. Glucose analogues did not modify the IRI release that was induced by a physiological concentration of glucose, even with CCK-8 (1 nM) (Fig. 1B). We confirmed that each pseudo-α-D-
glucose and 2DG alone did not show the insulinotropic activity in perfusion.

**Glucose analogues inhibit CCK-8-stimulated insulin release at high glucose concentration in perifused rat pancreatic islets**

Exposure of perifused islets to 500 nM CCK-8 with basal medium (2.8 mM) or a physiological concentration of glucose (5.6 mM) did not affect the basal rate of IRI release. In contrast, 100 nM CCK-8 at 11.1 mM glucose promptly increased IRI release both in the first and second phases (Fig. 2). These results are the same as those reported by Verspohl et al. (4). Furthermore, we investigated the effects of glucose analogues on glucose (11.1 mM)-induced IRI release with or without CCK-8 (100 nM).

The IRI level gradually rose by repeated stimulus with 11.1 mM glucose (Fig. 3A). When the 11.1 mM glucose-stimulated release was subtracted by the 2.8 mM glucose-stimulated release from the first stimulation, and this difference taken as 100%, the values for the second and third stimulation were 161 and 247%, respectively. Glucose (11.1 mM)-induced IRI release was remarkably decreased by 22.4 mM pseudo-α-D-glucose and 2DG (Fig. 3, B and C). The extents of inhibition by both glucose analogues were the same. Glucose-induced IRI release in the third stimulation recovered to the control values after washing out 2DG, but not after washing out pseudo-α-D-glucose.

The IRI release was augmented by repeated stimulus with CCK-8 (100 nM) plus glucose (11.1 mM) (Fig. 4A). When the 11.1 mM glucose-stimulated release was subtracted by the 2.8 mM glucose-stimulated release from the first stimulation, and this difference taken as 100%, the values for the second and third stimulation were 281 and 404%, respectively. 2DG (22.4 mM) inhibited CCK-8 (100 nM)-stimulated IRI release at 11.1 mM glucose. The extent of inhibition was 90.3% when compared with the IRI level by repeated stimulation with CCK-8 plus glucose (11.1 mM). The inhibition of IRI level in the third stimulation recovered to 64% after washing out (Fig. 4C). Pseudo-α-D-glucose (22.4 mM) inhibited IRI release by glucose (11.1 mM) plus CCK-8 when compared with the control. The IRI level was unchanged despite the absence of pseudo-α-D-glucose in the third stimulation (Fig. 4B). Pseudo-α-D-glucose and 2DG alone did not show the insulinotropic activity in perfusion.

**DISCUSSION**

There are many reports with regard to the cellular

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**Fig. 2.** The perifusion time course and extent of insulin release stimulated by glucose (11.1 mM, G11.1) plus 100 nM CCK-8 (closed triangles). The extent of insulin release is compared to that by 16.7 mM glucose (G16.7, open circles), and much greater than that by G11.1 alone (open triangles). The insulin release by 5.6 mM glucose (G5.6) plus 500 nM CCK-8 (closed squares) was low and not different from G5.6 alone (open squares). The islets were perifused totally for 160 min, initially at a low flow rate of 10 ml/hr for 60 min and at a basal rate of 1 ml/min for 60 min, and then stimulated with glucose alone or glucose plus CCK-8 for 40 min. Mean values of 3–7 experiments ± S.E.M. are given. All symbols (open and closed) show the IRI level per 1 min.
mechanisms of CCK-8-stimulated insulin release. CCK-8 that stimulates B cells in the basal state is probably released from the vagus nerve innervating islets because the intraportal administration of CCK-8 does not evoke insulin release at physiological concentrations (19). CCK-8 itself does not act on glucose utilization (6, 20), glucose phosphorylation and pentose phosphate shunt activity (20). CCK-8-stimulated insulin release depends upon ambient glucose levels and its accompanying metabolism. The insulinotropic effect of CCK-8 is dependent on extracellular Ca²⁺, suggesting that CCK-8 causes mobiliza-

tion of intracellularly stored Ca²⁺ (21). The insulinotropic effect of CCK-8 is partially mediated by the protein kinase C pathway (22). The lack of effect of CCK-8 on insulin release at a low glucose level (3.3 mM) may be explained by an insufficient activation of protein kinase C under these conditions (22). Therefore, the mechanisms of the glucose dependency of CCK-8-stimulated insulin release may be related to the interaction between the de novo synthesis of diacylglycerol accumulated by glucose (23, 24) and protein kinase C activated by CCK-8.

Glucose activates the glucokinase of pancreatic islets, in contrast to liver glucokinase (9). The glucokinase is a glucose-sensor in stimulus-secretion coupling (9–11). A

Fig. 3. Pseudo-α-D-glucose (PaG) (B) and 2-deoxy-D-glucose (2DG) (C) (22.4 mM) markedly inhibited glucose (11.1 mM, G₁₁₁) induced insulin release. A: G₁₁₁-induced insulin release without glucose analogues in perifused rat pancreatic islets. After perfusion at a low flow rate (10 ml/hr) for 60 min and then a basal rate (1 ml/min) for 60 min, the islets were perifused for a 10-min stimulation at the glucose concentrations indicated. The first stimulation was by G₁₁₁, the second one by G₁₁₁ with or without glucose analogues (22.4 mM), and the third one by G₁₁₁ alone. Columns show the period of each stimulation. Closed circles show the IRI level every 1 min. Dashed lines show the ending of stimulation and washing with basal medium (containing 2.8 mM glucose). The same results were obtained in 3–7 observations.

Fig. 4. Pseudo-α-D-glucose (PaG) (B) and 2-deoxy-D-glucose (2DG) (C) (22.4 mM) inhibited the insulin release by CCK-8 (100 nM) plus glucose (11.1 mM, G₁₁₁) in perifused rat pancreatic islets. A: Insulin release by CCK-8 plus G₁₁₁ without glucose analogues in perifused rat pancreatic islets. The first stimulation was by G₁₁₁ alone, the second one by CCK-8 (100 nM) plus G₁₁₁ with or without glucose analogues (22.4 mM), and the third one by CCK-8 plus G₁₁₁. Columns show the period of each stimulation. Closed circles show the IRI level every 1 min. Dashed lines show the ending of stimulation and washing with basal medium. The same results were obtained in 3–5 observations.
glucose analogue, pseudo-\(\alpha\)-DL-glucose inhibits islet glucokinase competitively with respect to \(d\)-glucose, and the concentration dependency of inhibition parallels in the inhibitory curve on glucose (10 mM)-induced insulin release (10). The other glucose analogue, 2DG is a metabolic inhibitor. 2DG is phosphorylated, and the phosphorylated intermediate inhibits phosphoglucose isomerase (\(d\)-glucose-6-phosphate ketol isomerase) (25, 26). In the present study pseudo-\(\alpha\)-\(d\)-glucose significantly inhibited high glucose (16.7 mM)-induced insulin release in perfusion, but not the normal glucose (5.6 mM)-induced one with or without CCK-8 (1 nM). The results indicate that at a normal glucose level, CCK-8 has little influence on the metabolic signals generated by glucose. Both glucose analogues inhibited the insulin response to glucose (11.1 mM), alone and in the presence of CCK-8 (100 nM), in perfusion. 2DG also showed the same results. The high levels of insulin release are essential for the inhibitory effects by glucose analogues either in perfusion or perifusion. The glucose dependency of CCK-stimulated insulin release at high glucose levels suggests that CCK-8 plays a physiological role to sustain glucose homeostasis. Furthermore, CCK may contribute to blood glucose lowering by releasing insulin glucose-dependently in non-insulin-dependent diabetes mellitus (NIDDM) where glucose-induced insulin release and the postprandial insulin release (especially the first phase) are decreased.

In conclusion, CCK-8-stimulated insulin release only at high glucose levels was glucose-dependent.

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