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Cholecystokinin (CCK_8) regulates glucagon, insulin, and somatostatin secretion from isolated rat pancreatic islets: interaction with glucose

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Abstract. The effect of CCK_8 on glucagon, insulin and somatostatin release and its interaction with glucose was studied in freshly isolated rat pancreatic islets. While glucose alone inhibited glucagon secretion [half-maximal effect (EC_{50}) = 4.6 mM], glucose in the presence of 10 nM CCK_8 increased glucagon release (EC_{50} = 6.9 mM). This effect of CCK_8 was dose-dependent at 11.1 mM glucose (EC_{50} = 1.0 nM). The dose-response curve for glucose on insulin secretion was shifted to the left by 10 nM CCK_8; the EC_{50} of glucose was 11.6 and 9.3 mM in the absence and presence of CCK_8, respectively. Glucose alone enhanced somatostatin release; this glucose-induced release was further increased by 10 nM CCK_8. Our data indicate that first, CCK_8 is able to reverse the inhibitory effect of glucose on glucagon secretion, second, CCK_8 sensitizes the beta cell to the insulinotropic effect of glucose, and third, CCK_8 enhances the effect of glucose on somatostatin release.

Key words: Cholecystokinin – Insulin secretion – Glucagon secretion – Somatostatin secretion

Introduction

Hormone secretion from the endocrine pancreas is under the regulation of nutrients, hormones and neurotransmitters (Porte and Halter 1981). Gut hormones such as GIP (glucose-dependent insulinotropic polypeptide) and CCK (cholecystokinin) are thought to play an important role as 'incretins' (Creutzfeld 1979; Szecówka et al. 1982). It has previously been shown that CCK interacts with specific CCK receptors on isolated rat pancreatic islets (Verspohl et al. 1986). However, clear dose-response curves for the interactions of CCK and glucose with respect to the secretion of the different islet hormones glucagon, insulin and somatostatin have not been shown. Those data are important since first the well known paracrine effects of each islet hormone (Patel et al. 1982) may be modulated by CCK and since second the effect of CCK on hormone release may be dissociated from that of glucose. It was, therefore, the aim of the present study to investigate whether CCK_8 solely increases the effect of glucose thereby sensitizing the endocrine pancreas for glucose or also modulates the effect of glucose on the secretion of islet hormones.

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Materials and methods

Animals. Wistar rats of either sex weighing between 180 and 250 g were used. They were kept on a standard pellet diet and tap water ad libitum at 22°C with a 12-h light dark cycle.

Chemicals. Synthetic CCK_8 was from Serva (Heidelberg, FRG). The following were purchased: pilocarpine hydrochloride, soybean trypsin inhibitor (SBTI), bacitracin, and Hepes from Sigma Chemical Co. (St. Louis, MO, USA); bovine serum albumin (BSA) fraction V from Miles Laboratories (Elkhart, IN, USA); (3-^{125}I iodotyrosyl^{11})Tyr^{11}-somatostatin-14, somatostatin antiserum from Amersham (Braunschweig, FRG); and collagenase (CLS grade) from Worthington Biochemicals Corp. (Freehold, NJ, USA). Insulin radioimmunoassay kits were supplied by Isotopendienst West GmbH (Dreieich, FRG). Rat insulin was purchased from Novo Research Institute (Copenhagen, Denmark). The glucagon kit including glucagon standards was from Serono (Freiburg, FRG).

Isolation of rat pancreatic islets and incubation conditions. Isolation of pancreatic islets was as described by Lacy and Kostianovsky (1967) and Kuo et al. (1973) with slight modifications (Verspohl and Ammon 1980). Three rats were each pretreated with 0.3 ml of 4% pilocarpine hydrochloride i.p. After 3 h, pancreata were isolated, minced, and washed twice with 20 ml ice-cold Hanks' solution containing 2.8 mM glucose, 1 mg/ml bacitracin, 0.2 mg/ml SBTI, and 0.02% albumin. Pancreas pieces were soaked and shaken in a 37°C water bath in the presence of 650 U collagenase/g tissue suspension. After 15–18 min of incubation the tissue suspension was transferred into 10 ml of ice-cold Hank's solution. Islets were separated by sedimentation and collected as described elsewhere (Lacy and Kostianovsky 1967). This method yields 200–400 islets/rat pancreas.

To measure hormone secretion, five islets were incubated for 60 min at 37°C in 1 ml Krebs-Ringer buffer plus 20 mM Hepes (KRH buffer), pH 7.4, containing 5 mg/ml bovine albumin, 1 mg/ml bacitracin and 0.2 mg/ml SBTI.

Determination of glucagon, insulin and somatostatin. Glucagon, insulin and somatostatin released into the medium by islets were assayed by radioimmunoassay kits using glucagon, rat insulin and somatostatin as standards, respectively. CCK_8 had been checked for noninterference with the insulin and glucagon radioimmunoassay.

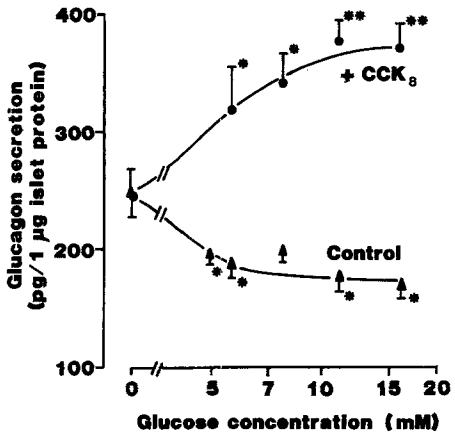

Fig. 1. Stimulation of glucagon release by glucose in the absence and presence of CCK. Ten islets were incubated at various glucose concentrations in the absence or presence of 10 nM CCK_8 for 60 min at 37° C. Results are expressed as pg glucagon secreted per µg islet protein over 60 min. Each value represents the mean ± SEM of four experiments (* $p < 0.05$, ** $p < 0.001$ vs. 0 glucose concentration)

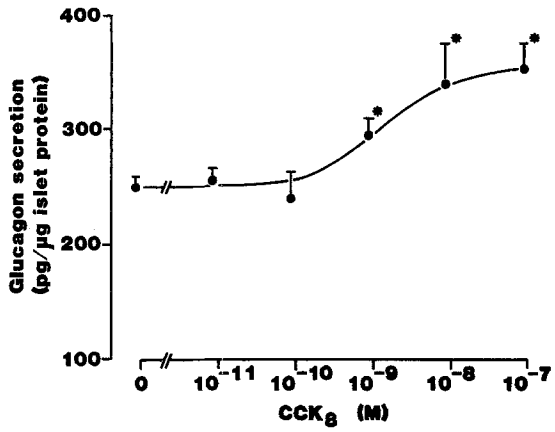

Fig. 2. Effect of CCK on glucose-modulated glucagon release. Ten islets were incubated with various CCK_8 concentrations in the presence of 11.1 mM glucose for 60 min at 37° C. Results are expressed as pg glucagon secreted per µg islet protein over 60 min. Each value represents the mean ± SEM of three experiments (* $p < 0.05$ vs. control)

In contrast to GIP (glucose-dependent insulinotropic peptide) (Siegel and Creutzfeldt 1985) or somatostatin (Turcot-Lemay et al. 1975) the CCK effect is well preserved on collagenase digested islets without having been precultured.

EC_{50}s of biologic effects were determined after logit-log transformation of data of each experiment (Ashton 1979). For statistical evaluation, multiple comparisons of means were carried out by two-way analysis of variance (F-test) and Students *t*-test.

Protein determination. The protein content of the solubilized pancreatic islets (solubilized with 0.1 N NaOH) was measured using bovine serum albumin as a standard (Bradford 1976).

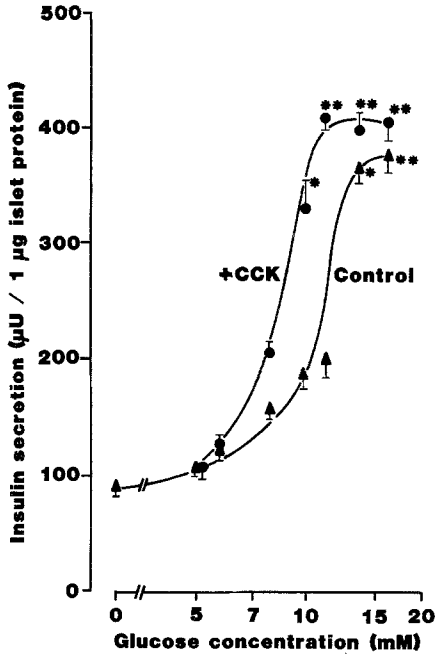

Fig. 3. Stimulation of insulin release by glucose in the absence and presence of CCK. Five islets were incubated at various glucose concentrations in the absence or presence of 10 nM CCK_8 for 60 min at 37° C. Results are expressed as µU insulin secreted per µg islet protein over 60 min. Each value represents the mean ± SEM of three experiments (* $p < 0.05$, ** $p < 0.01$ vs. 0 glucose concentration)

Results

Glucagon secretion

Glucose alone inhibited basal glucagon secretion in a concentration-dependent manner with a one-half maximal effect (EC_{50}) of 4.6 mM (Fig. 1). Addition of 10 nM CCK_8 reversed this glucose effect, i.e. in the presence of 10 nM CCK_8 glucose increased glucagon release in a concentration-dependent manner with an EC_{50} of 6.9 mM. As shown in Fig. 2 this synergistic effect of CCK_8 was concentration-dependent at 11.1 mM glucose; in this case its EC_{50} was 1.0 nM (Fig. 2).

Insulin secretion

Glucose alone stimulated insulin release in a concentration-dependent manner (Fig. 3). Addition of 10 nM CCK_8 shifted the dose response curve of glucose to the left without changing the maximal effect of glucose; the EC_{50} of glucose was decreased by 10 nM CCK_8 from 11.6 mM to 9.3 mM. As shown in Fig. 4 this effect was dependent on the CCK_8 concentration in the presence of 11.1 mM glucose.

Somatostatin secretion

Glucose alone stimulated somatostatin release in a concentration-dependent manner (Table 1, upper part). Addition of 10 nM CCK_8 shifted the somatostatin release to higher values; however, only at glucose concentrations higher than 8.3 mM this effect of CCK_8 was significant. When tested in the presence of 11.1 mM glucose the effect of CCK_8 was concentration-dependent (Table 1, lower part); the EC_{50} was 0.8 nM.

Table 1. Stimulation of somatostatin release by various glucose concentrations in the absence or presence of 10 nM CCK_8 and by various CCK_8 concentrations in the presence of 11.1 mM glucose. Rat pancreatic islets were incubated for 60 min at 37° C. Results are expressed as pg somatostatin secreted per µg islet protein over 60 min. Each value represents the mean ± SEM of three experiments. * $p < 0.005$ vs. data in the absence of CCK_8; ** $p < 0.05$ vs. 0 glucose concentration

	Glucose concentration (mM)				
	0	5	8.3	11.1	16.7
Control	14.7 ± 1.3	15.8 ± 0.2	16.4 ± 1.1	17.9 ± 0.6**	20.1 ± 1.1**
+ 10 nM CCK_8	17.7 ± 0.7	18.8 ± 0.9	16.9 ± 3.1	21.5 ± 1.0*, **	23.6 ± 1.1*, **

	CCK_8 concentration (nM)						
	0	0.01	0.1	0.32	1.0	10	100
+ 11.1 mM glucose	15.1 ± 0.8	13.9 ± 1.5	15.0 ± 0.9	16.8 ± 1.0	21.6 ± 1.1*	20.3 ± 0.8*	20.8 ± 0.8*

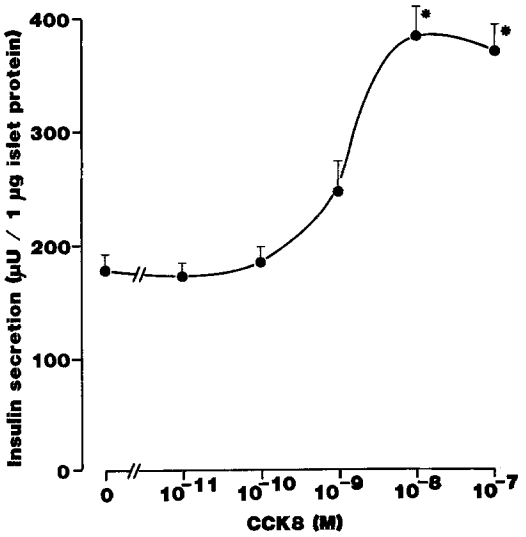

Fig. 4. Effect of CCK on glucose-modulated insulin release. Five islets were incubated with various CCK_8 concentration in the presence of 11.1 mM glucose for 60 min at 37° C. Results are expressed as µU insulin secreted per µg islet protein over 60 min. Each value represents the mean ± SEM of three experiments (* $p < 0.05$ vs. 0 glucose concentration)

Discussion

Our data clearly show that CCK_8 promotes the release of glucagon, insulin and somatostatin from rat pancreatic islets. Thus CCK may not only serve as an incretin by stimulating insulin secretion (Szećowka et al. 1982) and/or sensitizing the β-cell for the insulinotropic action of glucose (Szećowka et al. 1982), but also modifies the paracrine effects of hormones within the pancreatic islet.

Glucagonotropic effect. The primary physiologic role of glucagon is the preservation of normoglycemia that should be regulated at low glucose concentrations. Our data which are in accordance with those of Gerich et al. (1974) indicate that the A cell in fact is very sensitive to changes of glucose at low concentrations. This is evident from the fact that glucose decreases glucagon secretion with an EC_{50} as little as 4.6 mM. In comparison the EC_{50} of glucose for stimulation of insulin secretion is 11.6 mM.

Our data on the glucagonotropic effect of CCK are in line with those of others showing that CCK or caerulein (identical C-terminal sequence compared to CCK) enhance glucagon secretion in various species in vivo and in vitro (Ohneda et al. 1978; Williams and Champagne 1979; Hermansen 1980; Szećowka et al. 1982). It must be stressed that the combined effect of CCK_8 plus glucose on glucagon release differs from that of glucose alone in several ways: first, while glucose alone decreases glucagon secretion, its effect is reversed by CCK_8, second, the EC_{50}s of the inhibitory action of glucose due to the absence of CCK_8 and the stimulatory effect of glucose due to the presence of CCK_8 differ from each other.

CCK_8 stimulates glucagon release despite its stimulatory action on somatostatin and insulin secretion both of which have been reported to diminish the glucagon secretion (Östenson 1979; Mandarino et al. 1981) by their paracrine (indirect) action. Therefore, it appears that the effect of CCK_8 on glucagon secretion is rather a direct than an indirect one.

Insulinotropic effect. The effect of CCK_8 on insulin release depends on the presence of an insulin stimulatory concentration of glucose confirming similar data of others (Szećowka et al. 1982; Sakamoto et al. 1982; Okabayashi et al. 1983; Hermansen 1984). Thus, CCK_8 was not effective at a substimulatory glucose concentration. On the other hand, CCK_8 was ineffective during maximum stimulation of insulin release with high glucose concentrations. This latter observation is consistent with data on the mechanism of action of CCK_8 (Verspohl et al. 1987). Thus, in contrast to GIP (another incretin candidate; Siegel and Creutzfeldt 1985) CCK_8 is not able to increase the maximum amount of released insulin but rather increased the sensitivity of the B cell to submaximal stimulatory glucose concentrations usually appearing in the plasma after food intake.

Somatostatin releasing effect. Our data indicating that glucose increases somatostatin release are in line with those of Schauder et al. (1977). The effect of CCK_8 on somatostatin release appears not to be related to the presence of glucose since the dose response curves merely indicate an additive interaction of glucose and CCK_8. A specific role of CCK_8 in somatostatin secretion cannot be evaluated since the glucose-response curve is only slightly shifted upwards. The finding that the effect of CCK_8 on somatostatin release is rather weak is in line with data of others obtained from the perfused dog pancreas where CCK exhibited only a two-

fold and transient increase (Ipp et al. 1977). These data and our observation, however, do not fully rule out a role of somatostatin since in vivo the major part of CCK-mediated somatostatin release is from fundic mucosal cells (Soll et al. 1985) and other gastrointestinal sources.

Possible significance of glucagonotropic effect of CCK_8. In rats glucose alone has never been shown to be potent in mediating CCK release. However, after a mixed meal including fat and protein plasma CCK levels are increased (Liddle et al. 1984). Thus, after a carbohydrate-containing meal carbohydrates decrease plasma glucagon levels (Samols et al. 1983) whereas after a carbohydrate-free protein-rich meal, a low glucose concentration may be able to extraordinarily increase plasma glucagon levels as the result of increased plasma CCK levels (dual effect of glucose). Hypoglycemia after this sort of meal may be overcome by hepatic glucose production via CCK-mediated glucagon release thus getting the organism adapted to the sort of meal as may be speculated. By varying CCK release in response to the sort of meal, this might permit the gut to influence the rate at which glucose is either produced or eliminated from the circulation. It appears to be necessary to pay more attention to the glucagonotropic effects of incretins such as CCK. However, it has to be mentioned that these data on CCK effects may be different in other species, e.g. the pig.

In conclusion CCK_8 has a dose-dependent effect on insulin, glucagon and somatostatin release which are glucose-dependent except with respect to somatostatin release. Its effect on insulin release is direct. CCK_8 counteracts the inhibitory effect of glucose on glucagon release. It is speculated that the reversal by CCK_8 of the inhibitory effect of glucose on glucagon secretion may lead to a physiologic adaptation of the organism to the sort of meal which needs further investigations.

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