Participation of Glucokinase Inactivation in Inhibition of Glucose-Induced Insulin Secretion by 2-Cyclohexen-1-One

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We assessed our speculation that 2-cyclohexen-1-one (CHX) impairs glucose-induced insulin secretion through inactivation of glucokinase. Treatment of pancreatic islets with CHX at concentrations (0–5 mM) that caused a dose-dependent inactivation of glucokinase activity similarly inhibited glucose-induced insulin secretion. Another glucose-phosphorylating enzyme (hexokinase) in pancreatic islets was little affected by CHX. CHX-induced inactivation of glucokinase was blocked by the presence of its substrates (glucose and mannose) and an inhibitor (*N*-acetylglucosamine), all of which also protected against the inhibitory effect of the drug on glucose-induced insulin secretion. CHX also impaired insulin secretion induced by D-glyceraldehyde and dimethyl succinate, which are believed to stimulate the release of the hormone by being directly oxidized by glyceraldehyde-3-phosphate dehydrogenase, by entering the midstream of the glycolytic pathway as glyceraldehyde 3-phosphate, or by entering the tricarboxylic acid cycle in mitochondria after intracellular hydrolysis. The inhibitory effect of CHX on glucose-induced insulin secretion, however, was far more marked than that on insulin secretion evoked by D-glyceraldehyde and dimethyl succinate at any CHX concentrations used. Our study revealed that the inhibitory action of CHX on glucose-induced insulin secretion is exerted mainly, but not solely, through inactivation of glucokinase. This conclusion supports the view that glucokinase is a key enzyme in the recognition of glucose as an insulin secretagogue in pancreatic islets. *Diabetes* 39:1170–76, 1990

Reduced glutathione (GSH) levels in liver have been decreased by 2-cyclohexen-1-one (CHX), possibly by acting as a substrate of glutathione transferase (EC 2.5.1.18; 1). Later, it was found that CHX reacts nonenzymatically with GSH but not with cysteine (2). Some investigators have used CHX to study the role of GSH in cellular events, e.g., activation of polymorphonuclear leukocytes (2), amylase release from pancreatic acini (3), and insulin secretion from pancreatic islets (4). Sener et al. (4) reported that depletion of GSH in pancreatic islets by treatment with CHX impairs the ability of the islets to secrete insulin in response to nutrients including glucose. We felt uneasy, however, attributing the impairment of insulin secretion to the decrease in islet GSH, because Sener et al. (4) indicated that the inhibition of insulin release by CHX was dose dependent, but the depletion of GSH was not. The following reasons led us to speculate that CHX may inhibit glucose-induced insulin secretion by inactivating glucokinase. *1*) Because CHX has an activated double bond, the compound seems likely to react nonenzymatically with some protein-bound sulfhydryl groups, provided these groups are very nucleophilic. *2*) The enzyme glucokinase (high-K_m hexokinase, EC 2.7.1.1), which is thought to play a key role in glucose-stimulated insulin secretion (5–7), has a sulfhydryl group necessary for its activity (8). Our study assessed this speculation.

Liver glucokinase was shown to be kinetically and chromatographically indistinguishable from glucokinase purified from pancreatic islets (9). Because islet glucokinase is obtainable only in small amounts, we used glucokinase purified from liver instead of islet glucokinase in some of our experiments.

RESEARCH DESIGN AND METHODS

Partial purification of rat liver glucokinase and spectrophotometric assays of glucokinase and hexokinase (low-K_m hexokinase) were performed as described before (10). *N*-acetylglucosamine kinase (EC 2.7.1.59) was assayed by the method of Allen and Walker (11). Protein was assayed by

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the Bradford method with a kit from Bio-Rad (Richmond, CA), and bovine serum albumin was used as a standard. Livers were removed from fed male Sprague-Dawley rats (Clea Japan, Tokyo) anesthetized with pentobarbital sodium. The final preparation of glucokinase was dissolved in buffer A (pH 7), consisting of 20 mM potassium phosphate, 100 mM KCl, 1 mM $MgCl_2$, 1 mM EDTA, 0.1 mM dithiothreitol, 50 mM glucose, and 5% (vol/vol) glycerol. The enzyme preparation had a specific activity of 20 U/mg protein and contained no detectable activity of hexokinase or *N*-acetylglucosamine kinase.

Before the experiments on inhibition of glucokinase by CHX, a glucokinase stock solution was diluted 10-fold with glucose-free buffer A to lower the glucose concentration. The diluted enzyme solution (~4 U/ml) was used within 8 h. Ten microliters of the diluted enzyme solution was mixed with 80 μl of buffer B (dithiothreitol- and glucose-free buffer A), supplemented, if necessary, with sugar or dimethyl succinate. The mixture was preincubated for 1 min at 37°C, and incubation was started by adding 10 μl of a CHX solution (5–50 mM) to buffer B. CHX was stable in buffer B, so stock solutions of CHX were prepared only once per day. The reaction mixture of control experiments consisted of 10 μl of diluted glucokinase solution and 90 μl of buffer B. After incubation for the indicated period at 37°C, an aliquot (10 μl) of the reaction mixture was taken; glucokinase activity in it was measured in a total volume of 1 ml.

Pancreatic islets were isolated from fed male Sprague-Dawley rats weighing 300–350 g by a modification of the method of Hara et al. (12). Briefly, a collagenase solution (1.3 mg/ml) was injected into the bile duct, the distended pancreas was removed and incubated statically for 15 min at 37°C, and the digested tissue was filtered through three stainless steel meshes with pore sizes of 1190, 600, and 300 μm. Batches of five islets were placed in plastic cylinders (0.7 cm ID × 4.5 cm) covered with nylon mesh of 106-μm pore size at one end and preincubated for 30 min at 37°C in 1 ml buffer C (118 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 24.2 mM $NaHCO_3$, 0.2% bovine serum albumin, and 2.8 mM glucose [pH 7.4]) equilibrated with 5% CO_2/95% O_2. In time-course experiments on CHX inhibition of insulin secretion, however, preincubation was omitted. Preincubation and subsequent incubation were performed by placing each of the plastic cylinders in vials (1.4 cm ID × 4.2 cm) with flat bottoms. The islets were incubated for 30 min at 37°C in 1 ml buffer C (supplemented with CHX or CHX plus additive and equilibrated with 5% CO_2/95% O_2), washed for 1 min with buffer C at room temperature, and then incubated for 1 h at 37°C in 1 ml buffer C supplemented with 20 mM glucose, 10 mM D-glyceraldehyde, or 10 mM dimethyl succinate. In some experiments, islets treated with CHX were incubated in the presence of various concentrations of glucose or D-glyceraldehyde. In control experiments, the first incubation of islets was performed in buffer C only. Insulin secreted was assayed by enzyme immunoassay with a kit from Mitsui (Tokyo). Activities of glucokinase and hexokinase in pancreatic islets were assayed by fluorometry as described before (13).

Data were analyzed statistically by paired Student's *t* test with the level of significance set at $P < 0.05$.

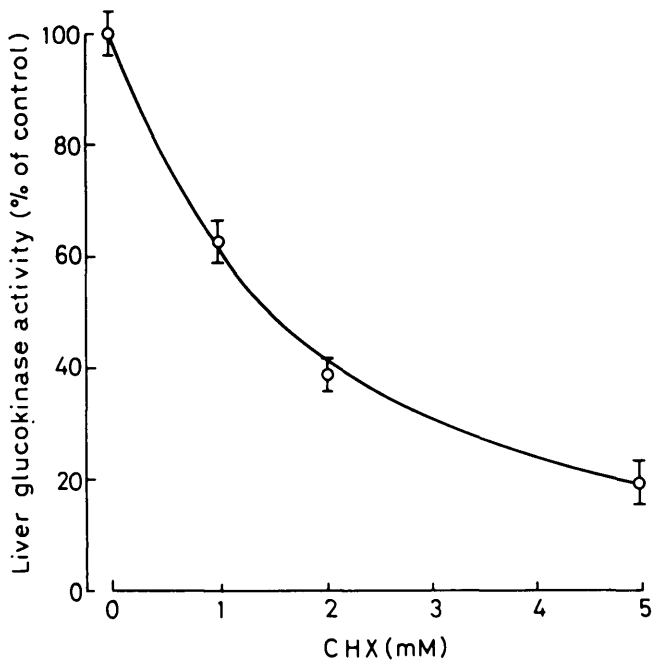

FIG. 1. Effect of 2-cyclohexen-1-one (CHX) concentration on liver glucokinase activity. Partially purified liver glucokinase was incubated with indicated concentrations of CHX for 30 min at 37°C; then residual activity of glucokinase was measured. Data are expressed as mean percentages ± SD of glucokinase activity (0.42 U/ml) of control experiments; *n* = 6.

RESULTS

Incubation of rat liver glucokinase with CHX (0–5 mM) for 30 min at 37°C caused concentration-dependent inhibition of the subsequently assayed glucokinase activity (Fig. 1). Inhibition of liver glucokinase by CHX was not released by dialysis. When pancreatic islets were treated with CHX (0–5 mM) for 30 min at 37°C, a similar concentration dependency was observed in inhibition of islet glucokinase by CHX (Fig. 2). Islet hexokinase, however, was not altered by CHX at any concentrations <5 mM.

Inhibition of liver glucokinase by CHX at 1, 2, and 5 mM was time dependent (Fig. 3). Impairment of glucose-induced insulin secretion by CHX was also time dependent (Fig. 4), and the time course was similar to that of inhibition of glucokinase activity. The abilities of various hexoses to protect against inhibition by CHX of liver glucokinase activity and glucose-induced insulin secretion are shown in Tables 1 and 2. Of the sugars used, glucose and mannose are substrates of glucokinase, *N*-acetylglucosamine is a competitive inhibitor of the enzyme, and 3-*O*-methylglucose and galactose are neither substrates nor inhibitors of it. The inhibition of glucokinase activity by CHX was attenuated by glucose, mannose, and *N*-acetylglucosamine but not by 3-*O*-methylglucose and galactose. The three sugars competent to protect against inhibition of glucokinase also blocked significantly the inhibition of insulin secretion by 0.5 and 2 mM CHX. Glucose at 20 mM completely protected insulin-secretory ability of islets from the inhibition by CHX at 0.5 mM. In addition, the order of potency of hexoses in preventing CHX inhibition of glucokinase activity (glucose>mannose >*N*-acetylglucosamine) was the same as that in blocking the CHX action on insulin secretion.

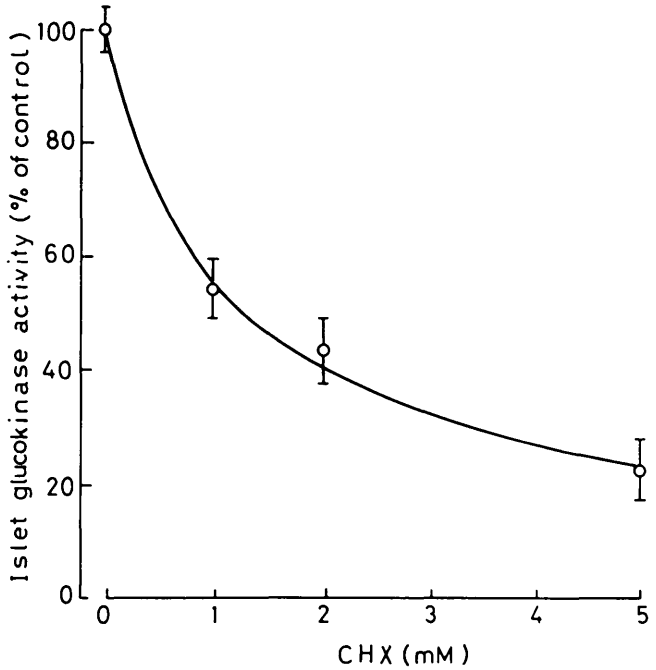

FIG. 2. Effect of 2-cyclohexen-1-one (CHX) concentration on islet glucokinase activity. Pancreatic islets were incubated with indicated concentrations of CHX for 30 min at 37°C and then assayed for glucokinase activity. Data are expressed as mean percentages ± SD of glucokinase activity (167 ± 7 pmol · h^{-1} · $islet^{-1}$) of control experiments; n = 5.

We also examined whether the insulin-secretory response of islets to nonhexose stimulants (e.g., glyceraldehyde and dimethyl succinate) is affected by CHX. The islet responses to nonglucose secretagogues were appreciably diminished by CHX (Figs. 5 and 6). We found, however, that the inhibitory effect of CHX on glucose-induced insulin secretion was far more profound than its inhibition of glyceraldehyde-induced

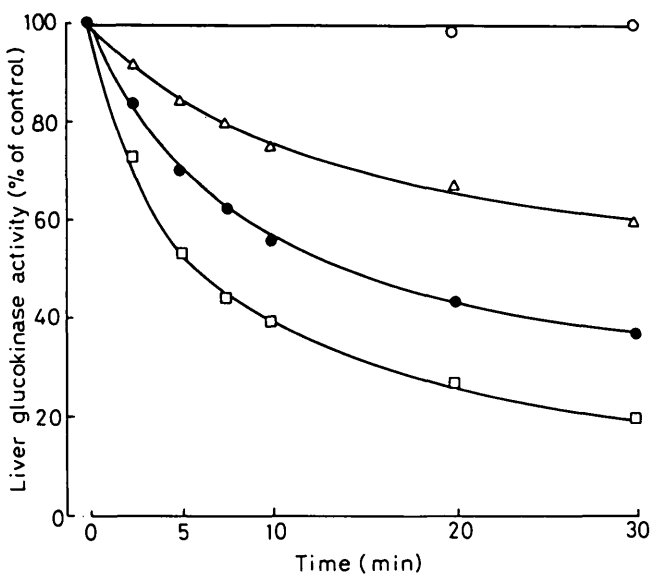

FIG. 3. Time courses of inhibition of liver glucokinase activity by 2-cyclohexen-1-one (CHX). Glucokinase was incubated at 37°C for various periods without CHX (○) or with CHX (△, 1 mM; ●, 2 mM; □, 5 mM) and then assayed for enzyme activity. Data represent means of 2 experiments; control activity was 0.39 U/ml.

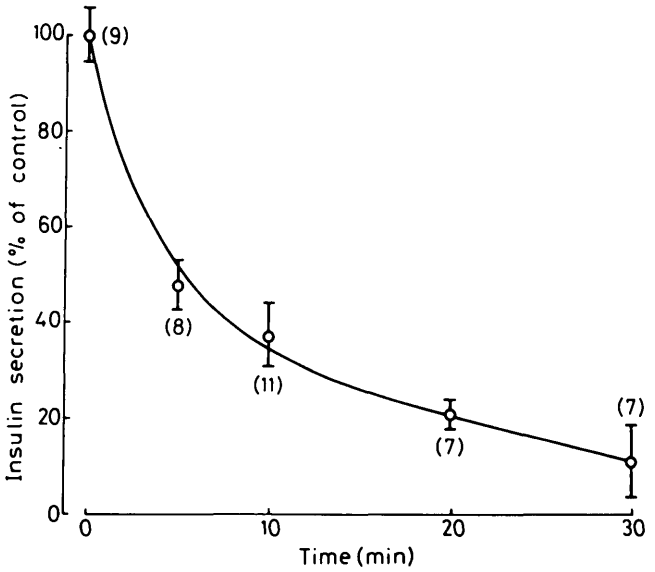

FIG. 4. Time course of inhibition of glucose-induced insulin secretion by 2-cyclohexen-1-one (CHX). Pancreatic islets were incubated with 2 mM CHX at 37°C for various periods and then assayed for insulin secretion induced by 20 mM glucose. Data are expressed as mean percentages ± SD of insulin secretion (2.07 ± 0.11 pmol · h^{-1} · $islet^{-1}$) of control experiments. (n).

and dimethyl succinate–induced insulin secretion at any CHX concentrations used (Fig. 7). At a concentration of 1 mM, for instance, CHX elicited ~80% inhibition of insulinotropism of glucose; on the other hand, it inhibited insulin-secretory responses to glyceraldehyde and dimethyl succinate only by 40 and 12%, respectively.

Inhibition of glyceraldehyde-induced and dimethyl succinate–induced insulin secretion by CHX was not protected by glucose and mannose (Table 3), both of which markedly blocked CHX inhibition of glucose-induced insulin secretion. On the other hand, the inhibitory actions of CHX against insulinotropism were completely protected by glyceraldehyde and dimethyl succinate. In addition, we examined whether the inhibitory actions of CHX against liver glucokinase activity and glucose-induced insulin secretion are prevented by glyceraldehyde or dimethyl succinate. Glyceraldehyde at 10 mM enhanced the inhibition of glucokinase activity by 2 mM CHX, whereas the inhibition of insulin secretion by CHX was not affected by glyceraldehyde (Table

TABLE 1
Effect of hexoses on inhibition of liver glucokinase activity by 2-cyclohexen-1-one (CHX)

Treatment	Percent of control
Control	100 ± 3
CHX	42 ± 2
+ Glucose	86 ± 2*
+ Mannose	72 ± 6*
+ *N*-acetylglucosamine	60 ± 6†
+ 3-*O*-methylglucose	44 ± 5
+ Galactose	43 ± 3

Liver glucokinase was incubated with 2 mM CHX at 37°C for 30 min in the presence or absence of 20 mM hexose and then assayed for the enzyme activity. Values are means ± SD of 3 experiments. *P < 0.001, †P < 0.01, vs. CHX alone.

TABLE 2
Effect of hexoses on inhibition of glucose-induced insulin secretion by 2-cyclohexen-1-one (CHX)

Treatment	0.5 mM CHX			2 mM CHX		
	n	Insulin secretion (pmol · h^{-1} · $islet^{-1}$)	Percent of control	*n*	Insulin secretion (pmol · h^{-1} · $islet^{-1}$)	Percent of control
Control	9	1.28 ± 0.12	100	8	1.43 ± 0.10	100
CHX	10	0.47 ± 0.08	37	8	0.08 ± 0.02	6
+ Glucose	8	1.17 ± 0.16*†	91	6	0.80 ± 0.12*	56
+ Mannose	8	0.99 ± 0.08*‡	77	7	0.33 ± 0.05*	23
+ *N*-acetylglucosamine	10	0.65 ± 0.09§	51	13	0.21 ± 0.04*	15
+ 3-*O*-methylglucose	8	0.44 ± 0.13	34	6	0.10 ± 0.02	7
+ Galactose	9	0.47 ± 0.08	37	5	0.08 ± 0.02	6

Pancreatic islets were incubated with 0.5 or 2 mM CHX at 37°C for 30 min in the presence or absence of hexose (20 mM) and then assayed for insulin secretion induced by 20 mM glucose. Insulin secretion is expressed as means ± SD.
*$P < 0.001$, §$P < 0.01$, vs. CHX alone.
†Not significantly different from control.
‡$P < 0.01$ vs. control.

4). Dimethyl succinate was ineffective in protecting against CHX inhibition of glucokinase activity and insulin secretion.

The inhibitory actions of 0.5 mM CHX on glucose-induced insulin secretion and 2 mM CHX on glyceraldehyde-induced insulin secretion were assayed at various concentrations of the respective secretagogues to examine the specificity of CHX action on both insulin-secretory responses. A similar experiment on dimethyl succinate–induced insulin secretion was not performed because of the low inhibitory activity of CHX on such a secretory response. In glucose-induced insulin secretion, sigmoidal dependence of insulin secretion on glucose concentration was observed not only in untreated islets but also in CHX-treated islets (Fig. 8); however, the glucose concentration for half-maximal insulin secretion ($S_{0.5}$ = 12 mM) and the Hill coefficient (n_H = 2.1) in untreated islets were different from the values ($S_{0.5}$ = 22 mM, n_H = 2.6) in CHX-treated islets. Glyceraldehyde-induced insulin secretion showed Michaelis-Menten hyperbolic reactivity (n_H = 1.0) in untreated islets, whereas weak sigmoidicity (n_H = 1.2) was observed in CHX-treated islets (Fig. 9). The K_m value (2.9 mM) in the former insulin-secretory response, however, was similar to that (2.5 mM) of the latter.

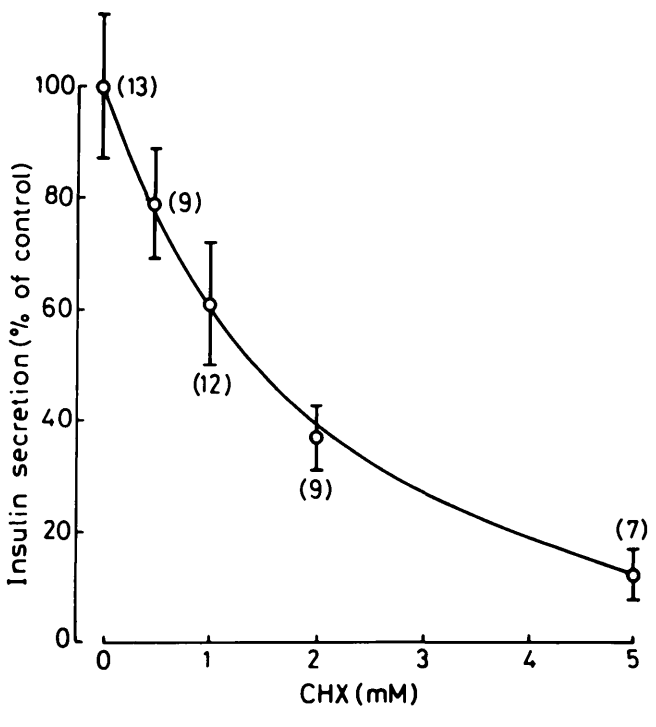

FIG. 5. Effect of 2-cyclohexen-1-one (CHX) concentration on glyceraldehyde-induced insulin secretion. Pancreatic islets were incubated with indicated concentrations of CHX for 30 min at 37°C and then assayed for insulin secretion induced by 10 mM D-glyceraldehyde. Data are expressed as mean percentages ± SD of insulin secretion (0.86 ± 0.10 pmol · h^{-1} · $islet^{-1}$) of control experiments. (*n*).

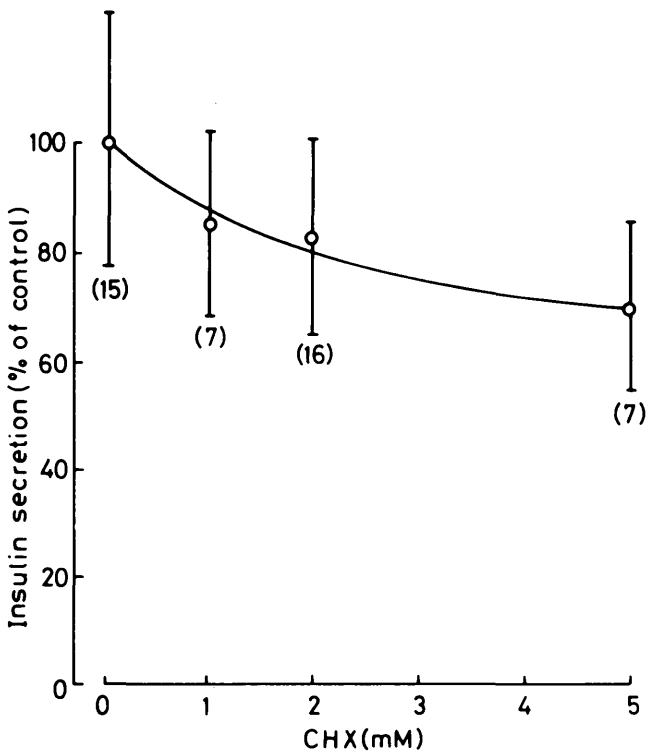

FIG. 6. Effect of 2-cyclohexen-1-one (CHX) concentration on insulin secretion induced by dimethyl succinate. Pancreatic islets were incubated with indicated concentrations of CHX for 30 min at 37°C and then assayed for insulin secretion induced by 10 mM dimethyl succinate. Data are expressed as mean percentages ± SD of insulin secretion (0.33 ± 0.07 pmol · h^{-1} · $islet^{-1}$) of control experiments. (*n*).

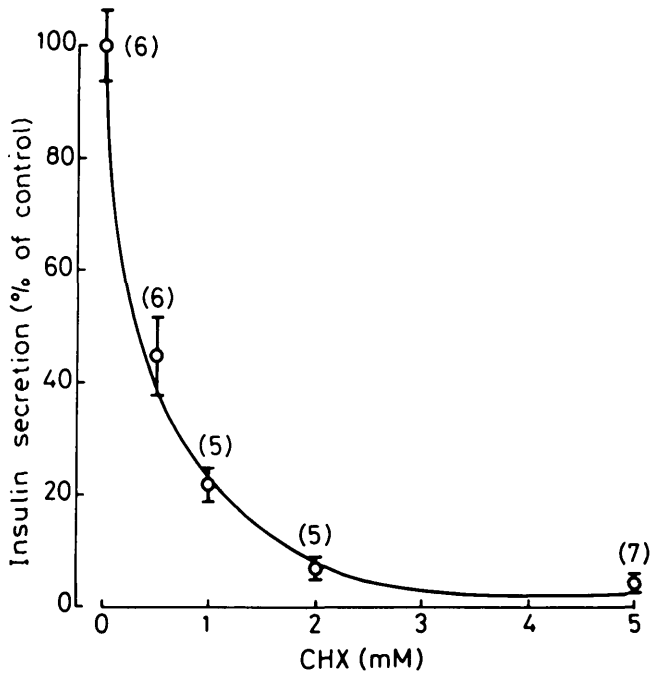

FIG. 7. Effect of 2-cyclohexen-1-one (CHX) concentration on glucose-induced insulin secretion. Pancreatic islets were incubated with indicated concentrations of CHX for 30 min at 37°C and then assayed for insulin secretion induced by 20 mM glucose. Data are expressed as mean percentages ± SD of insulin secretion (1.52 ± 0.10 pmol · h^{-1} · $islet^{-1}$) of control experiments. (*n*).

DISCUSSION

We first examined whether glucokinase is inhibited by CHX. The results indicated that CHX inhibits both liver and islet glucokinase. No release of the inhibition by dialysis revealed that the inhibition is irreversible. The data on protection of glucokinase by various hexoses from CHX inhibition suggested that CHX interacts with an essential sulfhydryl group at or near the substrate-binding site of glucokinase.

There was similar time dependency between CHX inhibition of glucokinase activity and CHX inhibition of glucose-induced insulin secretion. The protective abilities of various hexoses against CHX inhibition of glucokinase activity were also similar to those against CHX inhibition of glucose-induced insulin secretion. Thus, it is conceivable that glucokinase may be the primary site of CHX action in the inhibition of glucose-induced insulin secretion.

Glyceraldehyde was reported to stimulate insulin secretion by producing NADH with glycerate-1-phosphate by the action of glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12; 14) and by entering glycolysis after phosphorylation by triokinase (EC 2.7.1.28; 15,16). Dimethyl succinate was reported to stimulate insulin secretion by entering mitochondrial metabolism after its intracellular hydrolysis (17).

TABLE 3
Effect of insulin secretagogues on inhibition of glyceraldehyde-induced and dimethyl succinate–induced insulin secretion by 2-cyclohexen-1-one (CHX)

Treatment	Glyceraldehyde-induced insulin secretion		Dimethyl succinate–induced insulin secretion	
	n	pmol · h^{-1} · $islet^{-1}$	*n*	pmol · h^{-1} · $islet^{-1}$
Control	9	0.90 ± 0.19	7	0.31 ± 0.07
CHX	7	0.43 ± 0.07	16	0.24 ± 0.05*
+ Glucose	9	0.40 ± 0.06	8	0.23 ± 0.05*
+ Mannose	7	0.37 ± 0.05	12	0.22 ± 0.05*
+ Glyceraldehyde	9	0.86 ± 0.18*†		
+ Dimethyl succinate			7	0.38 ± 0.08*‡

Pancreatic islets were incubated with 2 mM CHX at 37°C for 30 min in the presence or absence of 20 mM glucose, 20 mM mannose, 10 mM D-glyceraldehyde, and 10 mM dimethyl succinate and then assayed for insulin secretion induced by 10 mM D-glyceraldehyde or 10 mM dimethyl succinate. Values are means ± SD.
*Not significantly different from control.
†$P < 0.001$, ‡$P < 0.01$, vs. CHX alone.

TABLE 4
Effect of glyceraldehyde and dimethyl succinate on inhibition of liver glucokinase activity and glucose-induced insulin secretion by 2-cyclohexen-1-one (CHX)

Treatment	Glucokinase activity (% of control)	Insulin secretion (pmol · h^{-1} · $islet^{-1}$)
Control	100 ± 3	1.27 ± 0.13
CHX	48 ± 2	0.10 ± 0.02
+ Glyceraldehyde	19 ± 1	0.12 ± 0.02
+ Dimethyl succinate	45 ± 3	0.11 ± 0.02

Liver glucokinase and pancreatic islets were incubated with 2 mM CHX at 37°C for 30 min in the presence or absence of 10 mM D-glyceraldehyde and 10 mM dimethyl succinate and then assayed for enzyme activity and insulin secretion induced by 20 mM glucose, respectively. Values are means ± SD of 3 and 6 experiments for glucokinase activity and insulin secretion, respectively.

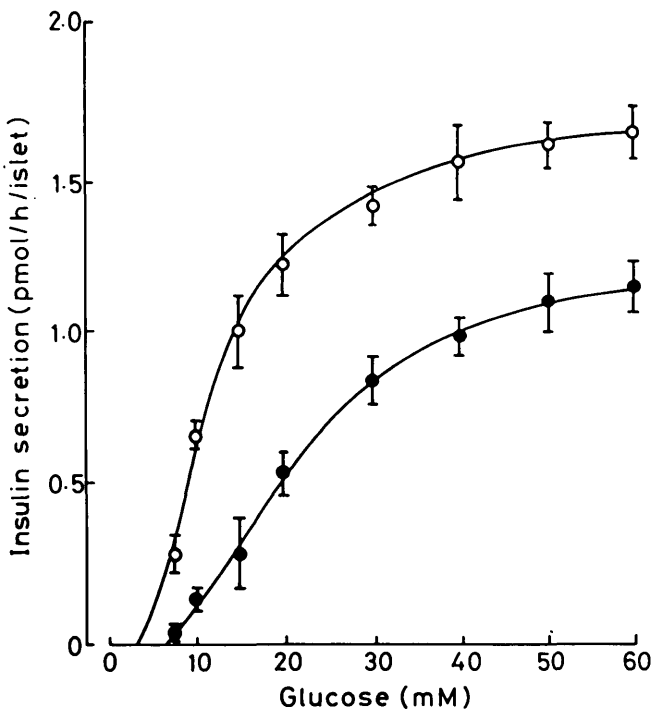

FIG. 8. Effect of glucose concentration on 2-cyclohexen-1-one (CHX) inhibition of glucose-induced insulin secretion. Pancreatic islets were incubated with (●) or without (○) 0.5 mM CHX for 30 min at 37°C and then assayed for insulin secretion induced by indicated concentrations of glucose. Data are expressed as means ± SD; *n* = 7–10.

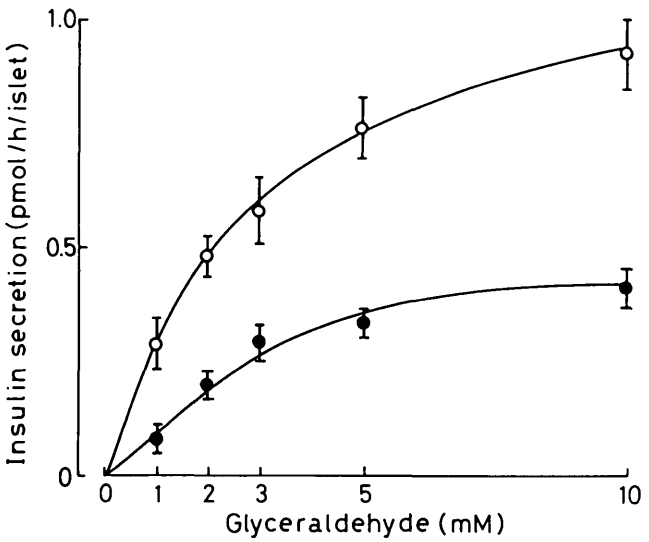

FIG. 9. Effect of glyceraldehyde concentration on 2-cyclohexen-1-one (CHX) inhibition of glyceraldehyde-induced insulin secretion. Pancreatic islets were incubated with (●) or without (○) 2 mM CHX for 30 min at 37°C and then assayed for insulin secretion induced by indicated concentrations of glyceraldehyde. Data are expressed as means ± SD; *n* = 6–8.

It might be expected that the insulinotropism of the two secretagogues might be little affected by CHX if the CHX action on glucokinase is highly specific. In fact, insulin-secretory responses of pancreatic islets to glyceraldehyde and dimethyl succinate (as well as glucose) were found to be impaired by CHX. This implies that some regulatory enzymes (e.g., in the glycolytic pathway, in mitochondrial metabolism) distal to glucose phosphorylation are also inhibited by CHX. We cannot exclude the possibility that the fall in islet GSH content by CHX is more or less responsible for the inhibition of insulin secretion by the drug (4). After all, the inhibitory effect of CHX on insulin secretion by nonhexose stimulants compared with secretion by glucose was markedly low. This supports the view that glucokinase can be considered the major site of CHX action; CHX affects not only glucokinase activity but also other regulatory reactions necessary for insulin secretion.

The discrepancy in CHX inhibition between the dose dependency of glucokinase activity (Fig. 2) and that of glucose-induced insulin secretion (Fig. 7) may be caused by the dependence of islet glucose usage on glucokinase activity throughout the physiological glucose concentration range (5). It has been reported that glucose-induced insulin secretion, as a function of islet glucose usage, shows a typical sigmoidal curve (18). Therefore, the extent of the loss of glucokinase activity cannot be directly proportional to that of the impairment of glucose-induced insulin secretion. It also appears that other CHX actions resulting in the depletion of GSH or the inhibition of some enzymes other than glucokinase contribute to make the inhibition of insulin secretion more severe. These explanations can be applied to the discrepancy between the protective potency of hexoses against the inhibition of glucokinase activity by 2 mM CHX (Table 1) and against the impairment of insulin secretion by 2 mM CHX (Table 2).

The glucose and mannose protection against CHX inhibition of glucose-induced insulin secretion may be caused by metabolism of the sugars resulting in maintenance of nearly normal levels of GSH and other metabolites in islets. If this were the case, glucose and mannose would be expected to protect glyceraldehyde-induced and dimethyl succinate–induced insulin secretion against inhibition by CHX. The two sugars, however, did not affect CHX inhibition of insulin secretion by nonhexose stimulants (Table 3). These results are compatible with the view that glucose and mannose block CHX inhibition of glucose-induced insulin secretion by binding to glucokinase competitively with CHX.

Complete protection against CHX inhibition of glyceraldehyde-induced insulin secretion by glyceraldehyde suggests that CHX possibly inhibits glyceraldehyde-3-phosphate dehydrogenase, which is a sulfhydryl enzyme (19), and accepts glyceraldehyde and glyceraldehyde 3-phosphate as substrates (14). We found that rabbit muscle glyceraldehyde-3-phosphate dehydrogenase was irreversibly inhibited by CHX, and the inhibitory potency of CHX against the enzyme was 25% of that against rat liver glucokinase (data not shown). Similarly, it is likely that dimethyl succinate blocks CHX inhibition of insulinotropism of the compound through the interaction of succinic acid, a hydrolytic product of dimethyl succinate, with succinate dehydrogenase (EC 1.3.99.1), a sulfhydryl enzyme (20).

Glyceraldehyde and dimethyl succinate were not effective in protecting glucokinase against CHX inhibition (Table 4), as was expected from the lack of relationship between the two compounds and the glucokinase reaction. We found, however, that the inhibition of glucokinase by CHX was fortified by the presence of glyceraldehyde. A preliminary experiment showed that glyceraldehyde irreversibly inhibits liver glucokinase (data not shown), resulting in more severe inhibition of glucokinase by CHX plus glyceraldehyde compared with CHX alone. The insulin-secretory response of islets treated with CHX plus glyceraldehyde was not different from that of islets treated with CHX alone, despite the marked difference in the effect on glucokinase activity between the two treatments. Perhaps the inhibition of insulin secretion by CHX alone is too severe to be further intensified.

It seems rational to think that dependency of insulin secretion on stimulant concentration in CHX-treated islets is not different from that in untreated islets if CHX targets only one of the reactions (or enzymes) responsible for insulin secretion by a stimulant. Furthermore, insulin-secretory responsiveness in CHX-treated islets should be lower than that in untreated islets for a wide range of stimulant concentrations. The results shown in Figs. 8 and 9, however, suggest that CHX does not specifically inhibit a single reaction (or enzyme) in glyceraldehyde-induced insulin secretion, because stimulant-concentration dependency of insulin secretion was altered by CHX treatment.

Our study indicates that the impairment of glucose-induced insulin secretion by CHX is attributable mainly, but not solely, to the inhibition of glucokinase. Our results agree with the concept that glucokinase plays a key role in the recognition of glucose as an insulin secretagogue in pancreatic islets.

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