Biochemical Pharmacology, Vol. 35, No. 21, pp. 3701–3708, 1986.
Printed in Great Britain.

0006–2952/86 $3.00 + 0.00
Pergamon Journals Ltd.

THE COUPLING OF METABOLIC TO SECRETORY EVENTS IN PANCREATIC ISLETS: EFFECTS OF 2-CYCLOHEXENE-1-ONE UPON GSH CONTENT AND SECRETORY BEHAVIOUR

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(*Received* 30 *January* 1986; *accepted* 8 *May* 1986)

Abstract—The GSH content and GSH/GSSG ratio were decreased in rat pancreatic islets exposed to 2-cyclohexene-1-one (CHX; 1.0 mM), but the drug failed to affect the cytosolic NADH/NAD^+ and NADPH/$NADP^+$ ratios. This coincided with inhibition of D-glucose oxidation, whilst the oxidation of L-leucine and L-glutamine was little affected by CHX (1.0 mM). The release of insulin evoked by either D-glucose or 2-ketoisocaproate was inhibited by CHX (1.0 mM), whereas such was not the case for insulin secretion induced by L-leucine, alone or in combination with L-glutamine. The latter amino acid protected the B-cell against the inhibitory action of CHX upon glucose-stimulated insulin release. CHX severely altered the normal relationship between nutrient oxidation, [^{45}Ca] net uptake and insulin release. Since CHX also inhibited insulin release evoked by non-nutrient secretagogues, it is speculated that GSH may be involved in several cytophysiological processes including the control of glycolysis, intracellular calcium distribution, and responsiveness to this cation of Ca^{2+}-sensitive targets.

It is currently believed that the capacity of nutrient secretagogues to stimulate insulin release is causally linked to an increase in B-cell respiration [1, 2], but the intimate nature of the coupling between nutrient metabolism and more distal events in the secretory sequence has yet to be fully elucidated [3]. We favour the view that such a coupling represents a multifactorial process [3, 4] including changes in the rate of generation of ATP [5], protons [6] and reducing equivalents [7, 8]. Each of these coupling factors might act on a distinct target system. In the present series of reports, we have so far examined the influence of nutrient secretagogues upon the cytosolic NADPH/$NADP^+$ ratio [9] and the possible role of glutathione reductase in the secretory response to such secretagogues [10]. Several carbamyl compounds, including 2-cyclohexene-1-one, decrease reduced glutathione (GSH) levels in liver or other tissues, possibly by acting as a substrate for glutathione *S*-transferase [11], and were used, therefore, as a tool to assess the role of glutathione in cell activation [12, 13]. In the present study, we have followed the same approach to investigate further the possible role of GSH in nutrient-stimulated insulin release.

MATERIALS AND METHODS

The drug 2-cyclohexene-1-one was purchased from Aldrich-Chemie (Steinheim, F.R.G.). All experiments were performed with islets isolated from the pancreas of fed albino rats [14]. The methods used to measure the release of insulin [14], oxidation of nutrients [15] and net uptake of [^{45}Ca] by the islets [16] and their content in GSH and GSSG [8], thiol [8], malate [9] and oxalacetate [17] were identical to those described in the cited references.

For measurement of the pyruvate content, groups of 50 islets each were, after incubation, mixed with 100 μl of perchloric acid (2.5%, v/v), placed in liquid nitrogen, and homogenized by mechanical vibration [7]. An aliquot (80 μl) of the islet extract was neutralized to pH 7–8 by mixing with 40 μl of Tris–KOH (0.2–1.0 M), and centrifuged for 3 min at 5000 *g*. Aliquots of 5 μl were used for the assay of malate, 50 μl for the assay of oxalacetate and 50 μl for the assay of pyruvate. In the latter case, 50 μl of the supernatant solution were mixed with 50 μl of a reaction mixture containing triethanolamine–HCl (100 mM, pH 7.0), NADH (1.0 mM), ammonium acetate (100 mM), ADP (1.0 mM) and beef liver glutamate dehydrogenase (EC 1.4.1.3; 0.1 μg/μl). After 20 min incubation at 22° to remove 2-ketoglutarate [9], the reaction was halted by heating for 5 min at 70° and the sample mixed with an equal volume (100 μl) of a second reaction mixture containing Tris–HCl (100 mM, pH 8.0), L-[1-^{14}C]glutamate (0.05 mM, 30 mCi/mmol) and pig heart glutamate-pyruvate transaminase (EC 2.6.1.2; 0.1 μg/μl). After 45 min incubation at 22°, the sample was diluted with 2.0 ml of iced H_2O and placed on a Dowex 50 (H^+ form) column so that the [1-^{14}C]2-ketoglutarate generated by transamination could be eluted by H_2O from the column and counted by liquid scintillation. The results were corrected for blank values found in the absence of islets, and expressed by reference to the readings obtained with standard amounts of pyruvate (12.5–100.0 pmol/sample) prepared in neutralized perchloric acid and treated in the same manner as the islet extracts. In this assay, the radioactivity eluted from the column in the absence of pyruvate represented no more than 1.6 ± 0.1% of the total radioactivity (N = 3), and

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Table 1. Effect of CHX upon islet glutathione content

Preincubation* (30 min)	Incubation (30 min)	GSH	GSSG	GSH/GSSG
CHX (mM)	D-glucose (mM)	(pmol/islet)	(pmol/islet)	(ratio)
Nil	Nil	8.97 ± 0.31 (28)	1.08 ± 0.31 (28)	8.39 ± 0.43 (28)
1.0	Nil	6.83 ± 0.26 (28)	1.06 ± 0.07 (28)	6.26 ± 0.42 (28)
2.0	Nil	6.85 ± 0.13 (7)	1.04 ± 0.04 (7)	6.45 ± 0.24 (7)
5.0	Nil	6.57 ± 0.28 (7)	1.02 ± 0.08 (7)	6.21 ± 0.15 (7)
Nil	16.7	10.46 ± 0.30 (32)	0.99 ± 0.06 (32)	10.75 ± 0.54 (32)
1.0	16.7	7.67 ± 0.51 (21)	0.90 ± 0.08 (21)	8.52 ± 0.72 (21)
2.0	16.7	8.20 ± 0.32 (12)	0.93 ± 0.03 (12)	8.65 ± 0.37 (12)

* The islets were always preincubated in the absence of D-glucose and incubated in the absence of CHX.

the recovery of pyruvate averaged 96.1 ± 8.4% and 99.1 ± 3.0%, respectively, in the ranges 0–37.5 and 37.5–100.0 pmol/sample (N = 9). The cytosolic NADH/NAD^+ and NADPH/$NADP^+$ ratios were judged, respectively, from the malate/oxalacetate and malate/pyruvate islet contents [18].

All results, including those just mentioned, are shown as the mean (±SEM) together with the number of individual observations (N), degree of freedom (d.f.), or statistical significance of differences between mean values as assessed by Student's *t*-test. The comparison between control and experimental data is restricted to results collected within the same experiment(s).

RESULTS

Effect of CHX upon glutathione content

In control islets not exposed to CHX, D-glucose (16.7 mM) increased ($P < 0.025$) both the GSH content and GSH/GSSG ratio (Table 1). When the islets were preincubated for 30 min in either the absence or presence of CHX (1.0–5.0 mM) and then incubated for another 30 min in the absence of the drug, CHX caused a significant fall in both the islet GSH content ($P < 0.001$ in all cases) and the GSH/GSSG ratio ($P < 0.005$ or less), whereas the GSSG content of the islets remained unaffected. A concentration of 1.0 mM CHX was apparently sufficient to cause a close-to-maximal fall in either the GSH content or GSH/GSSG ratio. Comparable effects of CHX were observed whether the final incubation of 30 min was carried out in the absence or presence of D-glucose (16.7 mM).

Effect of CHX upon cytosolic redox couples and thiol content

In the control islets not exposed to CHX, D-glucose (16.7 mM) augmented the malate and pyruvate content ($P < 0.001$) as well as the cytosolic NADH/NAD^+ and NADPH/$NADP^+$ ratios (Table 2), as judged from the malate/oxalacetate ratio ($P < 0.001$) and malate/pyruvate ratio ($P < 0.005$), respectively. In the absence of D-glucose, the results obtained in islets preincubated for 30 min with CHX (1.0 mM) were not significantly different from those obtained in control islets. In the presence of D-glucose, however, the pyruvate content was lower in CHX-pretreated than control islets ($P < 0.02$). Nevertheless, even in the glucose-stimulated islets, the redox state of the two cytosolic couples remained unaffected by CHX (Table 2).

D-Glucose increased the thiol content in control islets not exposed to CHX (Table 2, last line). Although CHX decreased the islet content in GSH, it failed to affect significantly the much higher total thiol content of the islets. Nevertheless, the response to D-glucose appeared somewhat more marked in control than CHX-pretreated islets. Indeed, the glucose-induced increment in thiol content averaged 31.6 ± 7.7 pmol/islet (d.f. = 25; $P < 0.001$) in control islets, as compared to only 18.2 ± 9.1 pmol/islet (d.f. = 25; $P < 0.06$) in CHX-pretreated islets.

Table 2. Effect of CHX upon cytosolic redox couples and thiol content

Preincubation (30 min)* CHX (mM)	Nil	1.0	Nil	1.0
Incubation (30 min) D-Glucose (mM)	Nil	Nil	16.7	16.7
Malate (fmol/islet)	709 ± 67 (15)	821 ± 101 (19)	1367 ± 99 (19)	1175 ± 106 (19)
Oxalacetate (fmol/islet)	9.43 ± 0.65 (19)	8.21 ± 0.56 (19)	9.95 ± 1.02 (19)	8.73 ± 0.72 (19)
Pyruvate (fmol/islet)	938 ± 70 (15)	914 ± 117 (15)	1387 ± 94 (15)	985 ± 131 (15)
Malate/oxalacetate (ratio)	79.2 ± 8.3 (19)	109.5 ± 14.5 (19)	156.9 ± 15.6 (19)	150.8 ± 16.8 (19)
Malate/pyruvate (ratio)	0.682 ± 0.061 (15)	0.733 ± 0.109 (15)	1.101 ± 0.096 (15)	1.243 ± 0.118 (15)
Thiol content (pmol/islet)	79.4 ± 4.9 (9)	81.9 ± 5.7 (10)	111.0 ± 4.8 (18)	100.1 ± 6.7 (17)

* The islets were always preincubated in the absence of D-glucose and incubated in the absence of CHX.

Table 3. Effect of CHX upon insulin release evoked by D-glucose

Incubation conditions*	Direct incubation†	Preincubation (no glucose)‡		
		No CHX	CHX (1.0 mM)	CHX (2.0 mM)
No glucose				
No CHX	15.8 ± 1.5 (19)§	22.5 ± 6.8 (18)		
Glucose (16.7 mM)				
No CHX	305.5 ± 11.5 (29)	220.2 ± 7.6 (44)	46.2 ± 4.0 (43)	12.2 ± 5.4 (18)
CHX (1.0 mM)	200.1 ± 11.8 (30)	146.4 ± 7.6 (26)		
CHX (2.0 mM)	104.8 ± 9.0 (37)			
CHX (5.0 mM)	12.5 ± 3.7 (20)			

* The concentrations of D-glucose and CHX during incubation are shown in the first column.
† The islets were directly incubated with or without D-glucose and/or CHX.
‡ The islets were preincubated for 30 min in the absence of glucose at the stated concentration of CHX, and then incubated for 90 min in the presence of D-glucose with or without CHX.
§ The release of insulin during incubation is expressed as μU/islet per 90 min.

Effect of CHX upon glucose-stimulated insulin release

When freshly isolated islets were incubated for 90 min, D-glucose (16.7 mM) augmented insulin output from a basal value of 15.8 ± 1.5 to 305.5 ± 15.8 μU/90 min per islet (Table 3). CHX (1.0–5.0 mM) caused a dose-related decrease in glucose-stimulated insulin release (Table 3, 2nd column), but failed to affect basal insulin output (see below, Table 5). At the highest concentration of CHX (5.0 mM), the output of insulin evoked by D-glucose was not different from basal release.

When the islets were preincubated for 30 min in the absence of glucose prior to being exposed to the hexose over the ensuing 90 min, the rate of insulin release evoked by D-glucose was lower than that observed in islets immediately incubated in the presence of the sugar (Table 3). After preincubation in the absence of D-glucose and during incubation in the presence of both D-glucose and CHX (1.0 mM), however, the relative extent of the inhibitory action of CHX upon insulin output was identical (33.5 ± 4.9% inhibition) to that observed in islets immediately submitted to incubation (34.5 ± 5.4% inhibition).

When CHX (1.0 mM) was present in the preincubation medium but absent during the subsequent incubation, the rate of insulin release was three times lower than that seen when CHX (also 1.0 mM) was absent during the preincubation and present only during the final incubation period. In other words, the relative extent of the inhibitory action of CHX (1.0 or 2.0 mM) was much more marked when the islets were pretreated with the drug prior to stimulation with D-glucose rather than being exposed simultaneously to CHX and the hexose.

Further experiments were designed in order to assess whether CHX affects the secretory response of the islets to other nutrient and non-nutrient secretagogues, and to explore whether it is possible to protect the islets against the inhibitory action of CHX.

Effect of CHX upon insulin output evoked by secretagogues other than D-glucose

The influence of CHX upon insulin release evoked by non-carbohydrate nutrients is illustrated in Table 4.

At a concentration of 1.0 mM, CHX inhibited

Table 4. Effect of CHX upon insulin release evoked by non-glucidic secretagogues

Incubation conditions*	Direct incubation†	Preincubation (no nutrient)‡		
		No CHX	CHX (1.0 mM)	CHX (2.0 mM)
No nutrient		9.5 ± 3.1 (20)§		
α-KIC (10 mM)				
No CHX	108.0 ± 2.9 (19)	81.3 ± 3.8 (20)	30.8 ± 3.0 (20)	
CHX (1.0 mM)	39.6 ± 4.9 (19)			
L-Leucine (15 mM)				
No CHX	53.1 ± 3.9 (30)	61.1 ± 3.3 (29)	90.3 ± 7.5 (29)	
CHX (1.0 mM)	73.4 ± 7.0 (30)			
L-Leucine (10 mM) + L-glutamine (10 mM)				
No CHX	321.1 ± 24.1 (19)	123.5 ± 9.5 (20)	216.8 ± 12.2 (20)	68.0 ± 11.7 (20)
CHX (1.0 mM)	347.1 ± 19.8 (19)			
CHX (2.0 mM)	130.9 ± 17.3 (19)			
CHX (5.0 mM)	13.3 ± 4.4 (19)			

* The concentrations of nutrient(s) and CHX during incubation are shown in the first column.
† The islets were directly incubated with or without nutrient(s) and/or CHX.
‡ The islets were preincubated for 30 min in the absence of nutrient at the stated concentration of CHX, and then incubated for 90 min in the presence of exogenous nutrient(s) but absence of CHX.
§ The release of insulin during incubation is expressed as μU/islet per 90 min.

Table 5. Effect of CHX upon insulin release evoked by non-nutrient secretagogues

D-Glucose (mM)	TPA (μM)	Gliclazide (μM)	Ba^{2+} (mM)	Ca^{2+} (mM)	Theophylline (mM)	Insulin output* (μU/islet per 90 min)	
						Control	CHX (2.0 mM)
—	—	—	—	1.0	—	15.8 ± 1.5 (19)	12.9 ± 1.0 (19)
5.6	—	61.9	—	1.0	—	47.2 ± 4.5 (19)	14.9 ± 1.4 (19)
2.8	0.2	61.9	—	1.0	—	249.0 ± 9.4 (18)	154.1 ± 11.5 (18)
—	—	—	2.0	—	1.4	217.5 ± 8.2 (18)	84.3 ± 9.6 (18)

* The islets were incubated for 90 min at the stated concentrations of secretagogue(s) and/or CHX.

insulin secretion provoked by 2-ketoisocaproate. The relative extent of such an inhibitory effect was the same whether the islets were immediately incubated in the presence of both the 2-keto acid and CHX (63.3 ± 5.3% inhibition), or first exposed for 30 min to CHX and then incubated for 90 min in the sole presence of 2-ketoisocaproate (62.1 ± 6.0% inhibition).

In sharp contrast to the results obtained with 2-ketoisocaproate, no inhibition of L-leucine-induced insulin release was observed when the islets were either immediately incubated in the presence of both the amino acid and CHX (1.0 mM) or first exposed to CHX (also 1.0 mM) and then stimulated by L-leucine. In both cases, the release of insulin was increased by CHX ($P < 0.02$ and $P < 0.001$). The relative magnitude of the enhancing action of CHX upon leucine-induced insulin release was also similar in both protocoles, with an overall mean value of 43.0 ± 10.1% (d.f. = 114).

When the combination of L-leucine and L-glutamine (10 mM each) was used to stimulate the islets, the rate of secretion was higher than that found in the presence of 2-ketoisocaproate or L-leucine. At a low concentration (1.0 mM), CHX again augmented insulin release, at least in islets first exposed to CHX prior to incubation in the presence of the two amino acids ($P < 0.001$). However, when the concentration of CHX was raised to 2.0 or 5.0 mM, a dose-related decrease in insulin output was observed in islets immediately incubated in the presence of both CHX and the two amino acids. Prior exposure of the islets to 2.0 mM CHX also caused inhibition of insulin release ($P < 0.001$) during a subsequent incubation in the presence of L-leucine and L-glutamine.

The results listed in Table 5 refer to the situation found with non-nutrient secretagogues. At a concentration of 2.0 mM, CHX abolished the release of insulin evoked, in the presence of 5.6 mM D-glucose, by the hypoglycemic sulfonylurea gliclazide. At the same concentration, CHX inhibited either the secretion of insulin provoked, in the presence of 2.8 mM D-glucose, by the combination of gliclazide and the tumor-promoting phorbol ester TPA or the hormonal release caused, in the absence of both glucose and Ca^{2+}, by the combination of Ba^{2+} and theophylline.

Protective effects of amino acids against the inhibitory action of CHX

The data presented in Table 4 led us to investigate whether amino acids may protect the B-cell against the inhibitory action of CHX upon glucose-stimulated insulin release.

Table 6. Effect of amino acids and NH_4Cl upon glucose-stimulated insulin release in the absence and presence of CHX

Experiment		Insulin output (μU/islet per 90 min)*	
1	L-Glutamine	Control	CHX (1.0 mM)
	Nil	297.1 ± 11.0 (18)	182.7 ± 8.6 (18)
	10.0 mM	299.1 ± 11.3 (18)	272.0 ± 9.3 (18)
2	L-Glutamine + glycine + L-cysteine	Control	CHX (2.0 mM)
	Nil	300.4 ± 12.3 (20)	100.8 ± 4.0 (20)
	2.0 mM each	277.7 ± 11.1 (20)	241.6 ± 13.0 (20)
3	L-Asparagine	Control	CHX (2.0 mM)
	Nil	304.4 ± 12.4 (18)	142.3 ± 10.6 (18)
	10.0 mM	286.7 ± 17.1 (18)	135.7 ± 10.7 (18)
4	L-Leucine	Control	CHX (2.0 mM)
	Nil	310.4 ± 13.7 (20)	107.4 ± 7.0 (20)
	10.0 mM	373.7 ± 11.4 (20)	221.0 ± 14.5 (20)
5	NH_4Cl	Control	CHX (2.0 mM)
	Nil	340.6 ± 12.8 (18)	148.9 ± 7.9 (18)
	0.25 mM	280.2 ± 12.3 (18)	114.0 ± 5.8 (18)

* The islets were always incubated for 90 min in the presence of D-glucose (16.7 mM) and, as indicated, CHX and/or the amino acid(s) or NH_4Cl.

Table 7. Effects of islet pretreatment with amino acid(s) and/or CHX upon the secretory response to D-glucose

Preincubation*	Control	CHX (1.0 mM)	CHX/Control
Nil	226.9 ± 5.3 (57)†	41.5 ± 3.5 (57)	18.3 ± 2.8%‡
L-Leucine (10 mM)	295.1 ± 8.2 (20)	51.6 ± 3.8 (20)	17.5 ± 3.1%
L-Glutamine (10 mM)	167.4 ± 12.0 (17)	70.5 ± 7.4 (17)	42.1 ± 8.4%
L-Leucine (10 mM) + L-glutamine (10 mM)	172.9 ± 16.0 (19)	124.7 ± 10.6 (20)	72.1 ± 11.1%

* The islets were preincubated for 30 min in the absence or presence of amino acid(s) with or without CHX, and then incubated for 90 min in the sole presence of D-glucose (16.7 mM).

† The release of insulin during incubation is expressed as μU/islet per 90 min.

‡ The release of insulin by CHX-pretreated islets is expressed relative to the mean corresponding control value, the SEM being now derived from those for both the control and experimental absolute secretory rates.

In a first series of experiments, the islets were immediately exposed for 90 min to D-glucose (16.7 mM), with or without CHX (1.0 or 2.0 mM), and in the absence or presence of the tested amino acid(s). In the absence of CHX, L-glutamine (10.0 mM) failed to affect significantly ($P > 0.8$) glucose-stimulated insulin release (Table 6, experiment 1). However, in the presence of CHX (1.0 mM), L-glutamine markedly augmented insulin output, which was now no more significantly different ($P > 0.05$) from that seen in the absence of CHX. Likewise, when the three amino acids forming the tripeptide glutathione were added to the incubation medium (each at a concentration of 2.0 mM), the rate of insulin release evoked by D-glucose in the absence of CHX was not significantly affected ($P > 0.1$), whereas the inhibitory action of CHX was considerably decreased (Table 6, experiment 2). Thus, in the presence of these three amino acids, the secretory rate was barely lower in the presence than absence of CHX ($P < 0.05$). L-Asparagine (10.0 mM) failed to mimic the protective action of L-glutamine, the secretory rate evoked by D-glucose being virtually identical in the absence or presence of L-asparagine, and this whether in the presence or absence of CHX (Table 6, experiment 3). L-Leucine (10.0 mM) significantly augmented glucose-induced insulin release in the presence of CHX ($P < 0.001$). However, L-leucine also augmented insulin release evoked by D-glucose in the absence of CHX ($P < 0.005$). It could thus be argued that the results obtained in the presence of L-leucine reflect its own secretory potential, rather than a protective effect of the branched chain amino acid. This interpretation is supported by the fact that CHX markedly inhibited ($P < 0.001$) insulin secretion evoked by D-glucose in the presence of L-leucine (Table 6, experiment 4). Since NH_4 accumulates in islets exposed to several of these amino acids [19, 20], we have also examined the effect of exogenous NH_4Cl (0.25 mM). Glucose-stimulated insulin release was inhibited by NH_4Cl, whether in the absence ($P < 0.005$) or presence ($P < 0.005$) of CHX (Table 6, experiment 5). The relative extent of the inhibitory action of NH_4Cl upon insulin release was not significantly different in the absence of CHX (17.7 ± 5.2% inhibition) and presence of CHX (23.4 ± 6.6% inhibition), respectively.

In the second series of experiments (Table 7), the islets were first preincubated for 30 min at 37° in the absence of glucose but presence of CHX and amino acid(s) and then further incubated for 90 min in the sole presence of D-glucose (16.7 mM). When L-leucine (10.0 mM) but not CHX was present in the preincubation medium, the amino acid significantly augmented ($P < 0.001$) the subsequent secretory response to D-glucose (Table 7). L-Leucine, however, failed to protect against the inhibitory action of CHX. Indeed, glucose-stimulated insulin release was inhibited, after exposure of the islets to CHX (1.0 mM), to the same relative extent whether L-leucine was present or not in the preincubation medium. In sharp contrast with the situation found in the islets first exposed to L-leucine, preincubation of the islets with L-glutamine (in the absence of CHX) inhibited the subsequent response to D-glucose ($P < 0.001$). However, the presence of L-glutamine in the preincubation medium significantly augmented insulin output from islets first exposed to CHX. As a result of these changes, the rate of secretion in CHX-pretreated islets, relative to the appropriate control value, was two times higher ($P < 0.001$) after preincubation in the presence of L-glutamine (42.1 ± 8.4% of control; d.f. = 32) than after preincubation in the absence of exogenous nutrient (18.3 ± 2.8% of control; d.f. = 112). When both L-glutamine and L-leucine, but not CHX, were present in the preincubation medium, the rate of insulin release evoked by D-glucose was again decreased ($P < 0.001$) to the same level as that found after exposure to L-glutamine alone. However, the presence of the two amino acids in the preincubation medium further augmented the secretory response to CHX-pretreated islets. Such a response, relative to its control value, was now four times higher than in islets first preincubated in the absence of amino acids.

Taken as a whole, these results suggest that certain amino acids, and especially L-glutamine, indeed protect the B-cell against the inhibitory action of CHX upon glucose-stimulated insulin release.

Effect of CHX upon nutrient oxidation

The data so far presented indicate that CHX lowers the GSH islet content, and that this effect, whilst not attributable to a change in cytosolic redox state, coincides with inhibition of insulin release. Further experiments were undertaken to assess whether CHX affects, in the islet cells, metabolic or functional variables other than insulin secretion.

In islets not submitted to any preincubation, CHX (1.0–5.0 mM) inhibited in a dose-related manner the

Table 8. Effect of CHX upon the oxidation of D-[U-^{14}C]glucose (16.7 mM)

Incubation conditions*	Direct incubations†	Preincubation (no glucose)‡		
		No CHX	CHX (1.0 mM)	CHX (2.0 mM)
No CHX	49.0 ± 2.0 (10)§	30.0 ± 1.9 (36)	10.5 ± 0.9 (31)	4.3 ± 0.7 (21)
CHX (1.0 mM)	25.1 ± 1.3 (10)	13.0 ± 1.1 (10)		
CHX (2.0 mM)	13.4 ± 1.0 (10)			
CHX (5.0 mM)	5.7 ± 0.7 (10)			

* The concentration of CHX during incubation is shown in the first column.

† The islets were directly incubated with D-[U-^{14}C]glucose at the stated concentration of CHX.

‡ The islets were preincubated for 30 min in the absence of glucose at the stated concentration of CHX, and then incubated for 90 min in the presence of D-[U-^{14}C]glucose with or without CHX.

§ The oxidation of D-[U-^{14}C]glucose during incubation is expressed as pmol/islet per 90 min.

oxidation of D-[U-^{14}C]glucose (Table 8). As little as 1.0 mM CHX was sufficient to inhibit glucose oxidation by 48.8 ± 4.9% (d.f. = 18, $P < 0.001$). When the islets were first preincubated for 30 min in the absence of D-glucose, the oxidation of D-[U-^{14}C]glucose (16.7 mM) during a subsequent incubation of 90 min appeared lower than that found in the first series of experiments in which the islets were not submitted to any preincubation. In the preincubated islets, the presence of CHX (1.0 mM) in the incubation medium inhibited D-[U-^{14}C]glucose oxidation to the same relative extent (56.6 ± 11.2%; d.f. = 18; $P < 0.001$) as that found in the first series of experiments (no preincubation). However, when CHX was incorporated solely in the preincubation medium, the relative extent of inhibition in D-[U-^{14}C]glucose oxidation during the final incubation appeared somewhat more marked than that seen when CHX was added solely to the incubation medium. Thus, whether in islets exposed to 1.0 or 2.0 mM CHX, the relative extent of inhibition in CHX-pretreated islets exceeded by 14.8 ± 6.4% (d.f. = 125; $P < 0.025$) the mean corresponding value recorded in islets immediately exposed to both D-[U-^{14}C]glucose and CHX. In other words, the relative extent of inhibition averaged, in CHX-pretreated islets, 127.2 ± 10.3% (d.f. = 89) of the mean corresponding value (100.0 ± 5.8%; d.f. = 36) found in the islets directly incubated in the presence of D-[U-^{14}C]glucose and CHX.

A somewhat different picture was obtained when the effect of CHX upon the oxidation of amino acids was examined (Table 9). The oxidation of L-[U-^{14}C]glutamine and that of L-[U-^{14}C]leucine (each 10.0 mM) were either measured in islets directly incubated in the presence of one of these amino acids and, as required, CHX (1.0–5.0 mM); or were assessed after 30 min preincubation of the islets with or without CHX (1.0 or 2.0 mM) and during a further 90 min incubation carried out in the simultaneous presence of the two amino acids and absence of CHX. In both cases, the relative extent of inhibition in oxidation rate was always less marked than that seen in the case of D-[U-^{14}C]glucose. Thus, when tested at a concentration of 1.0 mM, CHX failed to cause a significant fall in amino acid oxidation, whether in islets pretreated with CHX or in those directly exposed to the drug. Pooling the results obtained in these two procedures, the relative extent of inhibition averaged 15.4 ± 7.8% (d.f. = 38) in the case of L-[U-^{14}C]glutamine and 18.7 ± 14.9% (d.f. = 38) in the case of L-[U-^{14}C]leucine. However, when the concentration of CHX was raised to 2.0 mM, the inhibition was always significant ($P < 0.05$ or less) and averaged 37.0 ± 8.7% (d.f. = 38) in the case of L-[U-^{14}C]glutamine and 59.5 ± 12.8% (d.f. = 39) in the case of L-[U-^{14}C]leucine (pooled data obtained either during direct incubation or after preincubation with CHX). It should be noted that, in these experiments, the relative extent of inhibition was never

Table 9. Effect of CHX upon the oxidation of amino acids

CHX (mM)	Direct incubation*		Preincubated islets†	
	L-[U-^{14}C]Glutamine‡	L-[U-^{14}C]Leucine‡	L-[U-^{14}C]Glutamine + L-[U-^{14}C]Leucine‡	
Nil	83.1 ± 9.5 (10)	27.2 ± 2.3 (10)	52.9 ± 4.0 (11)	35.4 ± 6.1 (11)
1.0	67.5 ± 5.1 (10)	25.6 ± 5.3 (10)	46.4 ± 2.9 (11)	24.7 ± 3.6 (11)
2.0	42.1 ± 3.3 (10)	9.8 ± 2.7 (10)	39.2 ± 5.1 (11)	15.7 ± 4.0 (12)
5.0	23.6 ± 2.5 (9)	3.6 ± 1.2 (10)		

* The islets were directly incubated for 90 min in the presence of either L-[U-^{14}C]glutamine (10.0 mM) or L-[U-^{14}C]leucine (10.0 mM) at the stated concentration of CHX.

† The islets were preincubated for 90 min in the absence or presence of CHX (1.0 or 2.0 mM) in media deprived of exogenous nutrient, and then incubated for 90 min in the absence of CHX. The final incubation media contained both L-glutamine and L-leucine (10.0 mM each) together with a tracer amount of either L-[U-^{14}C]glutamine or L-[U-^{14}C]leucine.

‡ The oxidation rates are expressed as pmol/islet per 90 min.

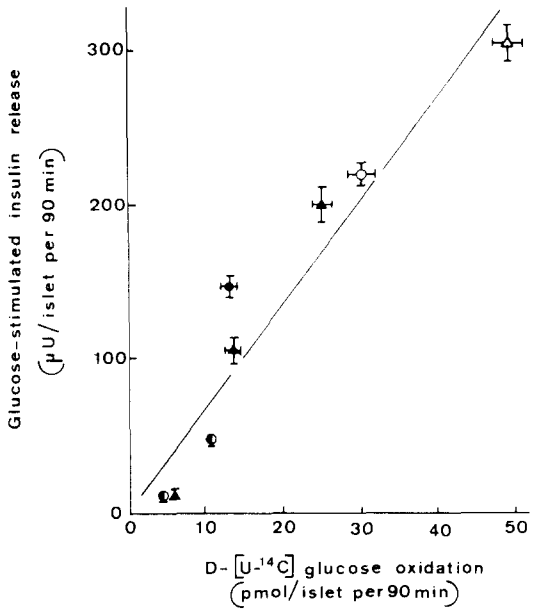

Fig. 1. Correlation between glucose-stimulated insulin release and D-[U-^{14}C]glucose oxidation in islets incubated for 90 min in the presence of the hexose (16.7 mM). In the first series of experiments, the islets were immediately incubated in the absence (open triangle) or presence (closed triangles) of CHX (1.0–5.0 mM). In the second series of experiments, the islets were either preincubated for 30 min in the absence of both CHX and D-glucose and then incubated for 90 min in the absence (open circle) or presence (closed circle) of CHX (1.0 mM), or preincubated in the presence of CHX (1.0 or 2.0 mM) and then incubated in the sole presence of D-glucose (half-filled circles). Mean values (±SEM) are taken from Tables 3 and 8.

significantly different in islets pretreated with CHX and in those directly exposed to the drug, respectively.

Figure 1 illustrates the correlation ($r = 0.9549$; $P < 0.001$) between the rates of D-[U-^{14}C]glucose oxidation and glucose-stimulated insulin release observed under eight distinct experimental conditions (data taken from Tables 3 and 8, respectively). A comparable correlation was not observed in the case of islets exposed to L-leucine and L-glutamine (compare Tables 4 and 9).

Effect of CHX upon [^{45}Ca]*uptake*

As illustrated in Table 10, CHX in a concentration of 1.0 mM tended to augment [^{45}Ca] net uptake by islets incubated in the presence of either D-glucose (16.7 mM) or the combination of L-leucine and L-glutamine (10.0 mM each). This unexpected enhancing trend failed, however, to achieve statistical significance ($P < 0.2$), except if the results obtained in the two series of experiments were pooled together in which case the enhancing action averaged 14.7 ± 6.7% of the mean corresponding control uptake (d.f. = 40; $P < 0.05$). The enhancing trend faded out when the concentration of CHX was raised to 2.0 mM, so that the absolute values for [^{45}Ca] net uptake were now virtually identical ($P > 0.6$ or more) to the mean control readings. At a still higher concentration (i.e. 5.0 mM), CHX severely decreased ($P < 0.001$) the net uptake of [^{45}Ca] by the islets exposed to either D-glucose or the two amino acids (Table 10).

DISCUSSION

The primary aim of the present experiments was to lower the GSH content in islet cells by use of CHX, and to examine the secretory consequence of such a biochemical manipulation. CHX indeed lowered the GSH content and GSH/GSSG ratio, whilst failing to affect the GSSG content of the islets. The fall in the GSH/GSSG ratio was not attributable to, and not accompanied by, any significant change in the cytosolic redox state of the NADH/NAD$^+$ and NADPH/NADP$^+$ couples, whether in islets deprived of exogenous nutrient or exposed to D-glucose. Incidentally, our results indicate that D-glucose increases the cytosolic NADH/NAD$^+$ ratio, in addition to its known effect to increase the cytosolic NADPH/NADP$^+$ [9, 21, 22]. The CHX-induced decrease in GSH islet content coincided with an impairment of insulin release evoked by either D-glucose or 2-ketoisocaproate. The inhibition by CHX of glucose-stimulated insulin secretion was apparently prevented by exposing the islets to L-glutamine, which was used either alone or in association with other amino acids such as those required for the synthesis of glutathione. All these data could be interpreted to indicate that a sufficient availability in GSH is required to allow nutrient secretagogues to exert their normal insulinotropic action in the pancreatic B-cell.

It is not evident, however, that the inhibition of glucose-stimulated insulin release by CHX is solely attributable to the fall in GSH islet content. Thus, even if such a fall were postulated to represent the primary effect of CHX, several of the present data

Table 10. Effect of CHX upon [^{45}Ca] net uptake

CHX (mM)	[^{45}Ca] net uptake (pmol/islet at 90th min)*	
	D-Glucose (16.7 mM)	L-Leucine + L-glutamine (10 mM each)
Nil	4.11 ± 0.25 (10)	4.76 ± 0.37 (12)
1.0	4.51 ± 0.13 (10)	5.65 ± 0.37 (12)
2.0	4.08 ± 0.22 (10)	4.99 ± 0.33 (12)
5.0	1.80 ± 0.09 (10)	2.34 ± 0.18 (12)

* The islets were incubated for 90 min in the presence of the stated nutrient(s) at the stated concentrations of CHX.

indicate that CHX did not perturbate solely the coupling of metabolic to secretory events in the islet cells. Indeed, CHX also inhibited glucose metabolism, as judged from the changes in both the pyruvate islet content and rate of D-[U-^{14}C]glucose oxidation. The oxidation of D-glucose was more severely affected than that of L-glutamine or L-leucine. Although CHX failed to cause any sizeable decrease in the thiol content of the islets, it is conceivable that the difference between the effect of CHX upon the oxidation of D-glucose and amino acids, respectively, reflects a preferential alteration of glycolysis, which involves enzymes with a cysteinyl thiol active center mechanism (e.g. glyceraldehyde-3-phosphate dehydrogenase). This interpretation is supported by the fact that 1,3-bis(2-chloroethyl)-1-nitrourea, a selective inhibitor of glutathione reductase, also affects more severely the oxidation of D-[U-^{14}C]glucose than that of either L-[U-^{14}C]leucine or L-[U-^{14}C]glutamine in pancreatic islets [10].

With this important reservation concerning the oxidation of nutrients in mind, the present data nevertheless suggest that CHX also affects more distal events in the secretory sequence. First, CHX impaired insulin release evoked by non-nutrient secretagogues such as a hypoglycemic sulfonylurea, a tumor-promoting phorbol ester or the association of Ba^{2+} and theophylline. In considering the latter findings, it should be realized, however, that CHX could conceivably alter the oxidation of endogenous nutrients and, by doing so, modulate the secretory response to these non-nutrient secretagogues [23]. Second and most importantly, CHX obviously perturbed both the coupling between nutrient oxidation and [^{45}Ca] net uptake and that between [^{45}Ca] uptake and insulin release. This was most striking in glucose-stimulated islets, although also evident in the islets exposed to L-glutamine and L-leucine. A possible interpretation of these data is that, in CHX-treated islets, the measurement of [^{45}Ca] uptake does not provide reliable information on the pool(s) of exchangeable calcium which is both regulated by the oxidation of nutrients and regulates the secretion of insulin [16]. Once again, a comparable, albeit not identical, situation was encountered in islets exposed to the inhibitor of glutathione reductase; in this case it was proposed that the drug alters the compartmentation of Ca^{2+} between mitochondrial (NADPH-dependent) and extramitochondrial (thiol-dependent) domains [10].

A multiple role of GSH in the metabolism of certain nutrients, the intracellular distribution of Ca^{2+} and, possibly, the response to this cation of Ca^{2+}-responsive targets, as suggested by both the present and a prior study [10], could well account for the contrasting effects of CHX upon the release of insulin evoked by distinct nutrients, e.g. 2-keto-isocaproate and L-leucine, even more so given that the normal secretory responses to these distinct nutrients already display distinct sensitivities to given alterations in Ca^{2+} fluxes [24, 25].

In conclusion, the present work supports the concept that GSH participates in several cytophysiological events involved in the process of stimulus–secretion coupling in the pancreatic B-cell.

Acknowledgements—This study was supported in part by grants from the Belgian Foundation for Scientific Medical Research and Ministry of Scientific Policy. We thank C. Rémion, J. Schoonheydt, M. Urbain and A. Tinant for technical help and C. Demesmaeker for secretarial assistance.

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