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THE COUPLING OF METABOLIC TO SECRETORY EVENTS IN PANCREATIC ISLETS: INHIBITION BY 2-CYCLOHEXENE-1-ONE OF THE SECRETORY RESPONSE TO CYCLIC AMP AND CYTOCHALASIN B

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Abstract—In rat pancreatic islets perifused in the presence of 2-cyclohexene-1-one (CHX; 1.0 mM), the secretory response to either D-glucose or 2-ketoisocaproate, but not that evoked by the association of L-leucine and L-glutamine, was severely decreased. This coincided with a decreased stimulation of [^{45}Ca] efflux from prelabelled islets, whereas the inhibitory action of D-glucose or 2-ketoisocaproate upon both [^{86}Rb] and [^{45}Ca] efflux appeared little or not affected. In the presence of D-glucose, the islets exposed to CHX were virtually unresponsive to either forskolin, theophylline or cytochalasin B. A severe decrease in the secretory response to forskolin was also observed in CHX-treated islets exposed to L-leucine and L-glutamine. Except for a somewhat lower sensitivity to NaF, no major change in adenylate cyclase activity or cyclic AMP production was observed in CHX-treated islets. The activity of protein kinase A was decreased in such islets but its responsiveness to cyclic AMP appeared unaltered. Transglutaminase activity was severely decreased in homogenates derived from CHX-treated islets. These findings suggest that CHX, possibly by lowering the GSH content of islet cells, impairs the functional capacity of the effector system for insulin release, in addition to and independently of any effect that it may exert upon nutrient catabolism and cationic fluxes in the islet cells.

In the preceding report in this series, we have proposed that 2-cyclohexene-1-one (CHX), by lowering the GSH content in rat pancreatic islets, affects both the oxidation of certain exogenous nutrients and intracellular distribution of calcium [1]. In addition, it was speculated that CHX might also alter the responsiveness to Ca^{2+} of Ca^{2+}-sensitive targets involved in the translocation and extrusion of secretory granules. The present study was undertaken in order to explore the validity of the latter concept. For such a purpose, we have mainly scrutinized the effects of CHX upon two functional variables, namely the fluxes of cations and the secretory response to insulinotropic agents thought to act at the level of the effector system for insulin release [2, 3].

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 [4].

The methods used to measure insulin release [5], [^{86}Rb] outflow [6] and [^{45}Ca] outflow [7] from prelabelled and perifused islets were identical to those described in the cited references. The nutrient-induced inhibition of [^{86}Rb] outflow was judged by paired comparison of the mean fractional outflow rate recorded over two periods of 5 min each, just prior to and after prolonged exposure to each nutrient. The nutrient-induced inhibition in [^{45}Ca] outflow was judged by paired comparison of the fractional outflow rates recorded just prior to and shortly after introduction of the nutrient. The secondary rise in [^{45}Ca] efflux evoked by nutrients was estimated from the difference between the lowest value for [^{45}Ca] fractional outflow rate reached shortly after introduction of the nutrient and the mean integrated value recorded over the ensuing period of stimulation. The nutrient-induced stimulation of insulin release was taken as the paired difference between mean basal value (measured in the last five samples prior to introduction of the nutrient) and the mean integrated secretory rate recorded during the entire period of exposure to the nutrient.

The methods used to measure the release of insulin [4] and production of cyclic AMP [8] by incubated intact islets and the activity of adenylate cyclase [3], protein kinase A [9] and transglutaminase [10] in islet homogenates were identical to those given in the cited references. The activity of transglutaminase was measured in the presence of *N*,*N*-dimethylcasein (1.0%, w/v), [2,5-^{3}H]histamine (0.5 mM) and Ca^{2+} (0.5 mM).

All results 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 values is restricted to results collected within the same experiment(s).

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Table 1. Basal cationic and secretory data

	No CHX	CHX (1.0 mM)
[^{86}Rb] FOR (%/min)		
Ca^{2+} 1.0 mM	3.99 ± 0.21 (14)	4.57 ± 0.26 (15)
No Ca^{2+}, EGTA 0.5 mM	4.24 ± 0.26 (16)	4.54 ± 0.26 (16)
[^{45}Ca] FOR (%/min)		
Ca^{2+} 1.0 mM	1.24 ± 0.09 (14)	1.32 ± 0.10 (15)
No Ca^{2+}, EGTA 0.5 mM	1.26 ± 0.13 (16)	1.16 ± 0.13 (16)
Insulin output (nU/min per islet)		
Ca^{2+} 1.0 mM	121 ± 25 (14)	143 ± 22 (16)

RESULTS

Basal cationic and secretory data

In order to investigate the effect of CHX upon the cationic and secretory response of the islets to either D-glucose (16.7 mM), 2-ketoisocaproate (10.0 mM) or the association of L-leucine and L-glutamine (10.0 mM), islets prelabelled with [^{45}Ca] and/or [^{86}Rb] were perifused for 70 min in the absence or presence of CHX (1.0 mM). The islets were deprived of exogenous nutrient for the first 45 min and then exposed to the secretagogue(s) for the last 25 min of perifusion. The fractional outflow rate (FOR) for either [^{86}Rb] and [^{45}Ca] and release of insulin were measured at normal extracellular Ca^{2+} concentration (1.0 mM). In order to obtain a reliable estimation of the inhibitory action of nutrient secretagogues upon [^{45}Ca] efflux, the [^{86}Rb] and [^{45}Ca] FOR was also measured in islets perifused in the absence of $CaCl_2$ and presence of EGTA (0.5 mM).

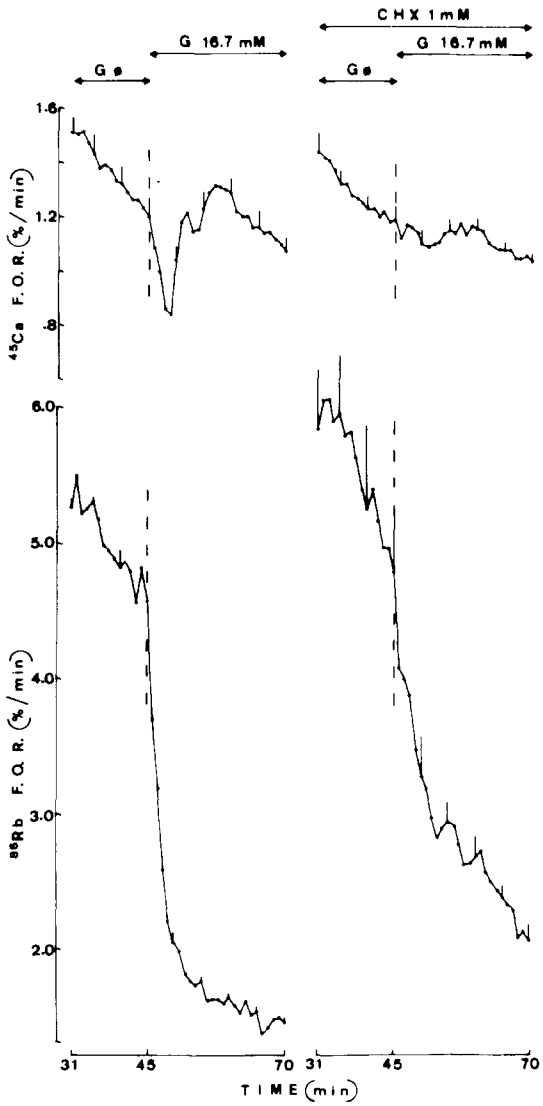

Fig. 1. Effect of D-glucose (16.7 mM) upon [^{45}Ca] and [^{86}Rb] outflow from islets perifused at normal extracellular Ca^{2+} concentration in the absence (left) or presence (right) of CHX (1.0 mM). Mean values (±SEM) refer to four experiments.

As documented in Table 1, CHX tended to slightly increase the basal [^{86}Rb] FOR. Such an increase only achieved statistical significance at normal Ca^{2+} concentration and when assessed by paired comparison ($P < 0.005$). The presence of CHX in the perifusate failed to affect the basal [^{45}Ca] FOR, whether in the presence or absence of Ca^{2+}. The basal insulin output was also unaffected by CHX.

Cationic and secretory response to D-*glucose*

Whether at normal Ca^{2+} concentration or in the absence of Ca^{2+}, D-glucose provoked a rapid and sustained decrease in [^{86}Rb] outflow (Fig. 1, lower panel). As judged from the paired difference in [^{86}Rb] FOR between the mean values recorded over the 5 min preceding the administration of D-glucose (min 41–45) and the last 5 min of exposure to the hexose (min 66–70), CHX failed to affect significantly the inhibitory action of the sugar upon [^{86}Rb] FOR (Table 2), whether in the presence or absence of extracellular Ca^{2+}.

In the absence of Ca^{2+}, glucose provoked a rapid and sustained decrease in [^{45}Ca] FOR. As judged from the paired difference between the value recorded just prior to the introduction of D-glucose (min 45) and that reached 4 min later (min 49), CHX failed to affect significantly the inhibitory action of the hexose upon [^{45}Ca] FOR (Table 2), the trend being towards a less marked effect of D-glucose in the presence than absence of CHX. At normal Ca^{2+} concentration, and in the absence of CHX, D-glucose provoked an initial fall in [^{45}Ca] outflow, this being soon followed by a secondary and biphasic increase in [^{45}Ca] FOR (Fig. 1, upper panel). CHX impaired, in two respects, this cationic response to D-glucose. First, the initial fall in [^{45}Ca] outflow appeared less marked (Table 2) and the lowest value for [^{45}Ca]

Table 2. Cationic and secretory response to D-glucose

	No CHX	CHX (1.0 mM)
Nutrient-induced decrease in [^{86}Rb] FOR (%/min)		
Ca^{2+} 1.0 mM	3.28 ± 0.06 (4)	2.89 ± 0.50 (4)
No Ca^{2+}, EGTA 0.5 mM	3.17 ± 0.40 (4)	3.26 ± 0.36 (4)
Nutrient-induced decrease in [^{45}Ca] FOR (%/min)		
Ca^{2+} 1.0 mM	0.36 ± 0.03 (4)	0.11 ± 0.03 (4)
No Ca^{2+}, EGTA 0.5 mM	0.25 ± 0.06 (4)	0.18 ± 0.01 (4)
Nutrient-induced increase in [^{45}Ca] FOR (%/min)		
Ca^{2+} 1.0 mM	0.37 ± 0.05 (4)	0.03 ± 0.01 (4)
Nutrient-induced increase in insulin output (nU/min per islet)		
Ca^{2+} 1.0 mM	580 ± 88 (4)	84 ± 39 (4)

FOR was reached 1 or 2 min later (min 50 or 51 instead of min 49) in the presence, as distinct from absence, of CHX. Second, the secondary rise in [^{45}Ca] outflow was severely impaired by CHX. Thus, as judged from the paired increment in [^{45}Ca] FOR between the lowest value reached shortly after introduction of D-glucose and the mean value recorded over the ensuing period of exposure to the hexose, the glucose-induced increase in [^{45}Ca] outflow was 10 times lower in the presence of CHX than in its absence (Table 2).

At normal Ca^{2+} concentration and in the absence of CHX, D-glucose provoked a biphasic increase in insulin output. The secretory response to the hexose was considerably decreased and apparently delayed in the presence of CHX (Fig. 2 and Table 2).

Cationic and secretory response to 2-ketoisocaproate

The influence of CHX upon the cationic and secretory response to 2-ketoisocaproate was largely comparable, albeit not identical, to that seen in the case of D-glucose. Both in the absence and presence of Ca^{2+}, 2-ketoisocaproate provoked a rapid and sustained decrease in [^{86}Rb] outflow, this phenomenon being unaffected by CHX (Fig. 3 and Table 3).

In the absence of Ca^{2+}, 2-ketoisocaproate caused a rapid and sustained decrease in [^{45}Ca] outflow. CHX failed to affect significantly this inhibitory action of 2-ketoisocaproate, the trend being towards a lesser decrease in [^{45}Ca] FOR in the presence than absence of CHX (Table 3). In the presence of Ca^{2+} but absence of CHX, 2-ketoisocaproate caused an early fall followed by a secondary and biphasic rise

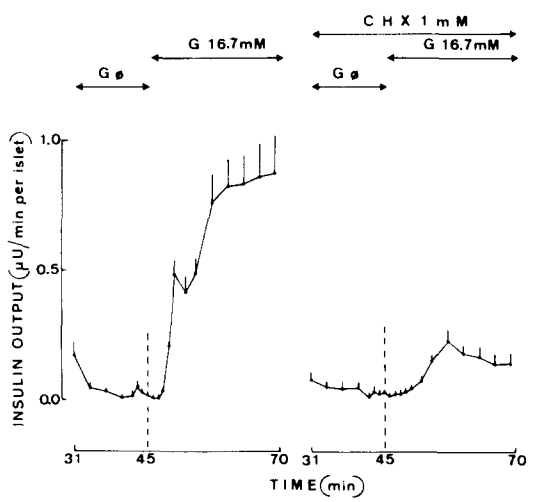

Fig. 2. Effect of D-glucose (16.7 mM) upon insulin release from islets perifused at normal extracellular Ca^{2+} concentration in the absence (left) or presence (right) of CHX (1.0 mM). Mean values (±SEM) refer to four experiments.

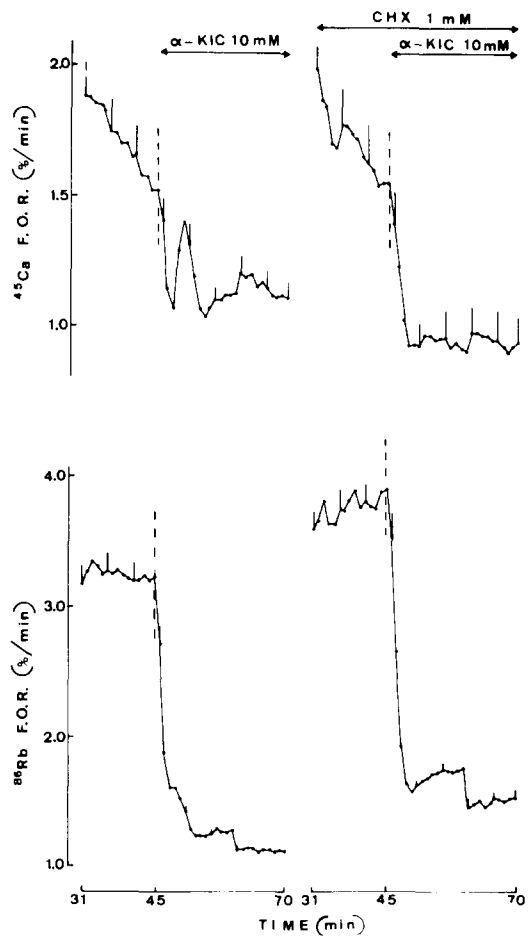

Fig. 3. Effect of 2-ketoisocaproate (α-KIC, 10.0 mM) upon [^{45}Ca] and [^{86}Rb] outflow from islets perifused at normal extracellular Ca^{2+} concentration in the absence (left) or presence (right) of CHX (1.0 mM). Mean values (±SEM) refer to 6–7 experiments.

Table 3. Cationic and secretory response to 2-ketoisocaproate

	No CHX	CHX (1.0 mM)
Nutrient-induced decrease in [^{86}Rb] FOR (%/min)		
Ca^{2+} 1.0 mM	1.98 ± 0.13 (6)	2.31 ± 0.11 (8)
No Ca^{2+}, EGTA 0.5 mM	3.05 ± 0.20 (6)	2.81 ± 0.15 (6)
Nutrient-induced decrease in [^{45}Ca] FOR (%/min)		
Ca^{2+} 1.0 mM	0.46 ± 0.06 (6)	0.64 ± 0.08 (7)
No Ca^{2+}, EGTA 0.5 mM	0.93 ± 0.06 (6)	0.76 ± 0.08 (6)
Nutrient-induced increase in [^{45}Ca] FOR (%/min)		
Ca^{2+} 1.0 mM	0.10 ± 0.04 (6)	0.05 ± 0.02 (7)
Nutrient-induced increase in insulin output (nU/min per islet)		
Ca^{2+} 1.0 mM	725 ± 76 (6)	244 ± 40 (8)

in [^{45}Ca] outflow (Fig. 3). The lowest value in [^{45}Ca] outflow reached shortly after introduction of the nutrient secretagogue and the early peak value reached during the secondary rise in [^{45}Ca] efflux occurred respectively 1 and 2 min earlier in the response to 2-ketoisocaproate than in response to D-glucose. In the presence of CHX and at normal Ca^{2+} concentration, the secondary rise in [^{45}Ca] efflux normally evoked by 2-ketoisocaproate was virtually abolished (Fig. 3). As a consequence, the lowest value for [^{45}Ca] FOR recorded shortly after introduction of the 2-keto acid was reached somewhat later (min 49–50 instead of min 48) and appeared somewhat lower, albeit not significantly so, in the presence of CHX than in its absence.

At normal Ca^{2+} concentration and in the absence of CHX, 2-ketoisocaproate provoked a biphasic stimulation of insulin release (Fig. 4). In good agreement with the cationic data, the early peak value for insulin release was recorded one min earlier in response to 2-ketoisocaproate than in response to D-glucose. CHX severely inhibited both the early and late components of the secretory response to 2-ketoisocaproate (Fig. 4 and Table 3).

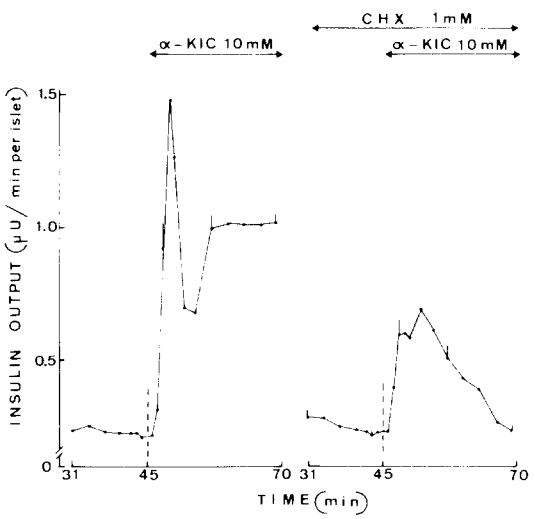

Fig. 4. Effect of 2-ketoisocaproate (α-KIC, 10.0 mM) upon insulin release from islets perifused at normal extracellular Ca^{2+} concentration in the absence (left) or presence (right) of CHX (1.0 mM). Mean values (±SEM) refer to 6–8 experiments.

Cationic and secretory response to L-*leucine and* L-*glutamine*

The simultaneous introduction of L-leucine and L-glutamine (10 mM each) provoked a rapid and sustained decrease in [^{86}Rb] FOR, whether in the absence or presence of Ca^{2+} (Fig. 5 and Table 4). CHX tended to accentuate such a fall in [^{86}Rb] FOR, the difference between control and experimental values being significant ($P < 0.05$) only in the presence of Ca^{2+} (Table 4). It should be noted, however, that the decrease in [^{86}Rb] FOR evoked by the amino acids displayed the same relative magnitude in the absence and presence of CHX, when expressed relative to the paired basal value. Thus, the amino acid-induced fall in [^{86}Rb] FOR in the presence of CHX and in its absence, respectively, corresponded to a relative decrease of 69.1 ± 3.0 and 57.2 ± 6.9% at normal Ca^{2+} concentration ($P > 0.1$) and 70.1 ± 1.9 and 66.5 ± 2.2% in the absence of Ca^{2+} ($P > 0.2$).

In the absence of Ca^{2+}, the association of the two amino acids provoked a rapid decrease in [^{45}Ca] outflow. At variance with the situation found in response to either D-glucose or 2-ketoisocaproate, this initial fall was followed by a slow reascension in [^{45}Ca] FOR (Fig. 5). The pattern and magnitude of the changes evoked by amino acids in the outflow of [^{45}Ca] from the calcium-deprived islets were similar in the presence or absence of CHX (Fig. 5 and Table 4). In the presence of Ca^{2+}, L-leucine and L-glutamine also provoked an early fall in [^{45}Ca] outflow followed by a secondary rise, the latter component being more marked in the presence than absence of extracellular Ca^{2+} (compare Figs 5 and 6). In the presence of Ca^{2+}, CHX failed to affect the amino acid-induced fall in [^{45}Ca] outflow but increased the magnitude of the secondary rise in effluent radioactivity (Fig. 6 and Table 4). Indeed, relative to the paired lowest value for [^{45}Ca] FOR reached shortly after the introduction of the amino acids, the mean increment in [^{45}Ca] FOR recorded over the ensuing period of stimulation corresponded to a relative increase of 21.5 ± 7.1% in the absence of CHX as distinct ($P < 0.05$) from 45.5 ± 6.5% in the presence of CHX.

The association of L-leucine and L-glutamine stimulated insulin release. The pattern of such a secretory response differed from that seen in response to either D-glucose or 2-ketoisocaproate by the fact that a progressive increase in insulin output was noticed throughout the 25 min of exposure to these amino acids. CHX provoked a dramatic

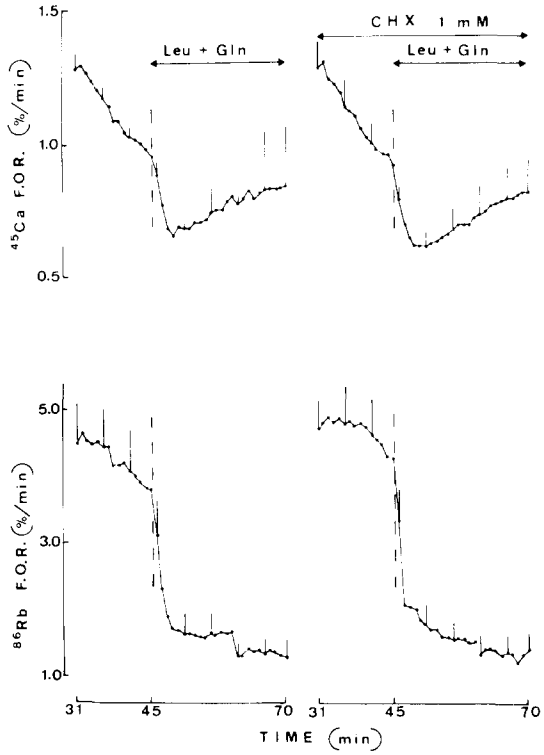

Fig. 5. Effect of the association of L-leucine and L-glutamine (10.0 mM each) upon [^{45}Ca] and [^{86}Rb] outflow from islets perifused in the absence of extracellular Ca^{2+} and presence of EGTA (0.5 mM), either in the absence (left) or presence (right) or CHX (1.0 mM). Mean values (±SEM) refer to six experiments.

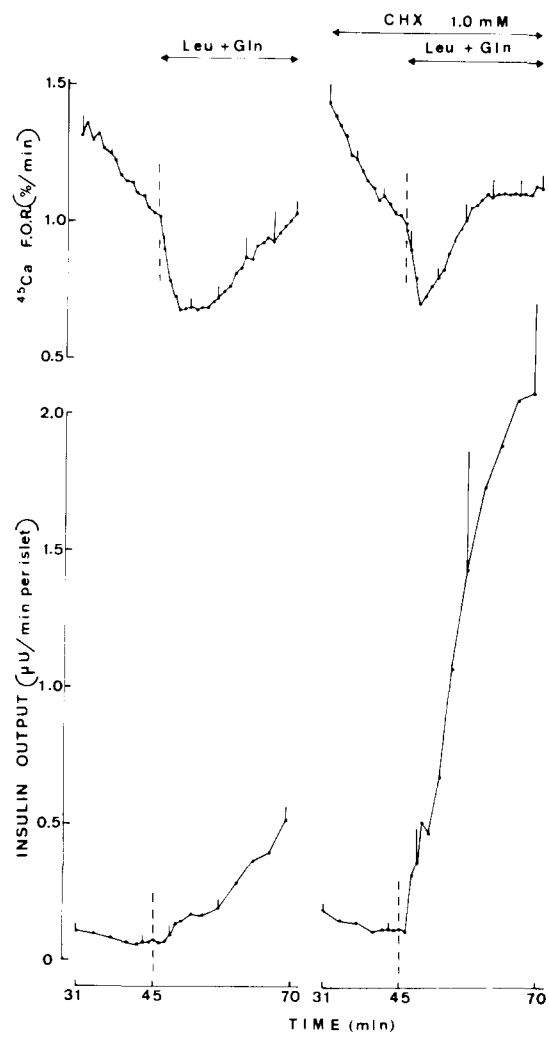

Fig. 6. Effect of the association of L-leucine and L-glutamine (10.0 mM each) upon [^{45}Ca] outflow and insulin release from islets perifused at normal extracellular Ca^{2+} concentration in the absence (left) or presence (right) of CHX (1.0 mM). Mean values (±SEM) refer to four experiments.

increase ($P < 0.03$) in the secretory response to such amino acids (Fig. 6 and Table 4), also in sharp contrast to the situation found with either D-glucose or 2-ketoisocaproate.

Effects of Ca^{2+}*, forskolin, theophylline and cytochalasin B upon insulin release*

CHX (1.0 mM) decreased by about one third, from 305.9 ± 11.4 to 200.1 ± 11.8 μU/islet per 90 min (N = 29–30), the release of insulin evoked by D-glucose (16.7 mM) in islets incubated for 90 min at 37°. The residual rate of insulin release evoked by D-glucose remained dependent on both the integrity of glucose metabolism and the availability of extracellular Ca^{2+}. Thus, in the presence of both D-glucose (16.7 mM) and CHX (1.0 mM), the rate of insulin output was further decreased from 209.6 ± 3.0 μU/90 min per islet to 17.5 ± 2.2 μU/90 min per islet when mannoheptulose (15 mM) was added to the medium and to 27.3 ± 3.0 μU/90 min per islet (N =

Table 4. Cationic and secretory response to L-leucine and L-glutamine

	No CHX	CHX (1.0 mM)
Nutrient-induced decrease in [^{86}Rb] FOR (%/min)		
Ca^{2+} 1.0 mM	2.51 ± 0.29 (4)	3.73 ± 0.37 (4)
No Ca^{2+}, EGTA 0.5 mM	2.60 ± 0.37 (6)	3.08 ± 0.37 (6)
Nutrient-induced decrease in [^{45}Ca] FOR (%/min)		
Ca^{2+} 1.0 mM	0.35 ± 0.03 (4)	0.30 ± 0.02 (4)
No Ca^{2+}, EGTA 0.5 mM	0.31 ± 0.04 (6)	0.32 ± 0.04 (6)
Nutrient-induced increase in [^{45}Ca] FOR (%/min)		
Ca^{2+} 1.0 mM	0.17 ± 0.06 (4)	0.32 ± 0.05 (4)
Nutrient-induced increase in insulin output (nU/min per islet)		
Ca^{2+} 1.0 mM	178 ± 43 (4)	1148 ± 324 (4)

Table 5. Effect of CHX upon glucose-stimulated insulin release

D-Glucose (mM)	Forskolin (μM)	Theophylline (mM)	Cytochalasin B (μM)	Insulin output*	
				No CHX	CHX (2.0 mM)
16.7	—	—	—	311.6 ± 12.2 (57)	106.9 ± 7.1 (58)
16.7	10.0	—	—	647.2 ± 9.7 (19)	94.3 ± 9.5 (19)
16.7	—	1.4	—	671.2 ± 34.6 (10)	114.0 ± 8.1 (29)
16.7	—	—	20.9	712.9 ± 32.4 (9)	113.4 ± 8.3 (28)

* The output of insulin was measured over 90 min incubation in the absence or presence of CHX, and is expressed as μU/islet per 90 min.

12 in all cases) when the medium was deprived of $CaCl_2$ and enriched with EGTA (0.5 mM).

However, in contrast to control islets, those exposed to CHX (2.0 mM) were unable to display any increase in glucose-stimulated insulin output when either forskolin (10.0 μM), theophylline (1.4 mM) or cytochalasin B (20.9 μM) was added to the incubation medium (Table 5).

Since certain amino acids, especially L-glutamine, may protect the islet cell against the deleterious effect of CHX, we have examined the effect of the latter drug upon the secretory response to forskolin in islets exposed to L-glutamine and L-leucine (10 mM each). Forskolin (10 μM) doubled insulin release evoked, in the absence of CHX, by the association of L-leucine and L-glutamine (Table 6). Although CHX, at a concentration of 1.0 mM, failed to affect significantly the secretory response to these amino acids, it virtually abolished the enhancing action of forskolin. At a higher concentration (2.0 mM), CHX caused a 68.9 ± 10.1% inhibition of insulin release evoked by the two amino acids, and again impaired the enhancing action of forskolin. Pooling the data obtained at the two concentrations of CHX (1.0 and 2.0 mM), the relative magnitude of the enhancing action of forskolin averaged 29.3 ± 13.5% ($P < 0.05$; d.f. = 54), as distinct ($P < 0.001$) from 105.8 ± 9.4% ($P < 0.001$; d.f. = 24) in the absence of CHX. These data indicate that, even in the presence of L-leucine and L-glutamine, CHX affects preferentially the response to forskolin as distinct from that evoked by these amino acids.

Adenylate cyclase activity and cyclic AMP production

The findings illustrated in Tables 5 and 6 led us to investigate whether the activity of enzymes involved in the functional response of islet cells to cyclic AMP and/or Ca^{2+} was altered in CHX-treated islets. In this perspective, we have first examined the effect of CHX upon both adenylate cyclase activity in islet homogenates and cyclic AMP production by intact islets.

Preincubation of the islets for 30 min with CHX (1.0 or 2.0 mM) failed to cause any obvious alteration of basal adenylate cyclase activity in islet homogenates (Table 7) and failed to affect adversely the

Table 6. Effect of CHX upon amino acid-stimulated insulin release

L-Leucine (mM)	L-Glutamine (mM)	Forskolin (μM)	CHX (mM)	Insulin output* (μU/islet per 90 min)
10.0	10.0	—	—	294.7 ± 25.7 (12)
10.0	10.0	10.0	—	606.5 ± 13.0 (14)
10.0	10.0	—	1.0	281.2 ± 25.7 (14)
10.0	10.0	10.0	1.0	343.8 ± 31.5 (14)
10.0	10.0	—	2.0	91.6 ± 14.8 (14)
10.0	10.0	10.0	2.0	124.8 ± 15.5 (14)

* The output of insulin was measured over 90 min incubation in the absence or presence of CHX (1.0 or 2.0 mM).

Table 7. Effect of CHX upon adenylate cyclase activity

Assay conditions	No CHX*	CHX (1.0 mM)	CHX (2.0 mM)
Basal	348 ± 6 (3)†	315 ± 8 (3)	338 ± 5 (3)
NaF (10 mM)	1162 ± 11 (3)	644 ± 17 (3)	809 ± 11 (3)
	[3.34 ± 0.04]‡	[2.05 ± 0.06]	[2.39 ± 0.04]
Forskolin (10 μM)	1499 ± 16 (3)	1406 ± 21 (3)	1581 ± 39 (3)
	[4.31 ± 0.05]	[4.46 ± 0.07]	[4.67 ± 0.12]

* The islets were preincubated for 30 min in the absence or presence of CHX (1.0 or 2.0 mM) prior to being homogenized for measurement of enzymic activity.

† The reaction velocity is expressed as amol/islet per min.

‡ The reaction velocity is expressed relative to basal value (in brackets).

Table 8. Effect of CHX upon cyclic AMP net production

D-Glucose (mM)	Theophylline (mM)	No CHX*	CHX (1.0 mM)
1.7	1.4	41.4 ± 2.2 (19)†	47.8 ± 3.0 (17)
16.7	—	23.6 ± 3.9 (17)	22.1 ± 4.5 (17)
16.7	1.4	68.7 ± 3.7 (26)	64.5 ± 5.7 (25)

* The islets were incubated for 60 min in the absence or presence of CHX .1.0 mM).

† The cyclic AMP content of the islets and incubation medium is expressed as fmol/islet.

response to forskolin (10 μM). However, in the presence of NaF (10.0 mM), both the absolute reaction velocity and its value relative to basal activity were significantly lower ($P < 0.001$) in islets preincubated with CHX (1.0 or 2.0 mM) than in control islets. The relative magnitude of the CHX-induced impairment in the responsiveness of adenylate cyclase to NaF was not vastly different in islets first exposed to either 1.0 or 2.0 mM CHX. Thus, when the reaction velocity recorded in the presence of NaF was expressed relative to the paired value found in the presence of forskolin, the NaF/forskolin ratio was not significantly different ($P > 0.4$) in islets pretreated with either 1.0 mM CHX (45.8 ± 4.2%) or 2.0 mM CHX (51.2 ± 5.0%), both values being significantly lower ($P < 0.01$) than that recorded in the control islets (77.5 ± 1.7%).

In the control islets incubated for 60 min at 37° in the presence of D-glucose (16.7 mM), theophylline (1.4 mM) increased three-fold ($P < 0.001$) the amount of cyclic AMP recovered in the islets and incubation medium (Table 8). In the presence of theophylline, a rise in D-glucose concentration from 1.7 to 16.7 mM also increased ($P < 0.001$) the net production of cyclic AMP. CHX (1.0 mM) failed to affect significantly the net production of the nucleotide. The capacity of theophylline to increase the amount of cyclic AMP was of comparable magnitude in CHX-treated and control islets. The enhancing action of D-glucose (16.7 mM), albeit still significant ($P < 0.05$) in the CHX-treated islets, appeared somewhat lower, however, than in control islets, at least when expressed relative to the paired value found at low concentration of D-glucose ($P < 0.025$ for the comparison between control and CHX-treated islets).

Protein kinase A activity

When islets were preincubated for 30 min with CHX (1.0 mM) and then homogenized for the measurement of protein kinase activity, both the basal and cyclic AMP-activated reaction velocities were lower ($P < 0.02$) than in control islets preincubated in the absence of CHX (Table 9). Cyclic AMP, in the presence of theophylline, enhanced the reaction velocity in both control and CHX-treated islets ($P < 0.025$), the relative magnitude of such an enhancing action being virtually identical in both cases ($P > 0.95$).

Transglutaminase activity

When islets were preincubated for 60 min in either the absence or presence of CHX (1.0 mM) and then homogenized for measurement of transglutaminase activity, the reaction velocity averaged in the CHX-treated islets no more than 10.3 ± 5.3% ($N = 3$; $P < 0.005$) of the paired control value (10.0 ± 2.8 pmol/islet per 60 min). No inhibition of enzymic activity was found when CHX was added directly to the assay medium in a concentration (0.1 mM) exceeding that which could be attributed to contamination of the islets preincubated with CHX, these being washed twice prior to homogenization.

DISCUSSION

The present data indicate that CHX does not affect the capacity of nutrient secretagogues to cause a rapid and sustained decrease in [^{86}Rb] outflow from prelabelled islets perifused in either the absence or presence of extracellular Ca^{2+}. This finding is not incompatible with the knowledge that the catabolism

Table 9. Effect of CHX upon protein kinase A activity

Assay conditions	No CHX*	CHX (1.0 mM)
Basal	1.025 ± 0.077 (7)†	0.693 ± 0.035 (7)
Cyclic AMP (0.1 mM) and theophylline (2.5 mM)	1.422 ± 0.127 (7)	0.965 ± 0.092 (7)
Cyclic AMP/basal (%)‡	138.7 ± 12.4 (7)	139.3 ± 13.2 (7)

* The islets were preincubated for 30 min in the absence or presence of CHX (1.0 mM) prior to being homogenized for measurement of enzymic activity.

† The reaction velocity is expressed as pmol/islet per 5 min.

‡ The paired ratio between cyclic AMP-activated and basal activity is expressed as a percentage.

of certain of these nutrients, especially D-glucose, is impaired in CHX-treated islets [1]. Thus, a maximal decrease in [^{86}Rb] outflow is reached at concentrations of nutrient secretagogues well below those required to cause a maximal secretory response [11]. Hence a partial decrease in nutrient catabolism may coincide with a normal inhibition of [^{86}Rb] outflow when nutrient secretagogues are used at concentrations of high insulinotropic efficiency.

The fact that CHX impaired the capacity of glucose to decrease [^{45}Ca] outflow (especially in the presence of extracellular Ca^{2+}), to provoke a secondary rise in [^{45}Ca] outflow and to stimulate insulin release could indeed reflect the alteration of glucose metabolism by CHX [1]. However, in response to stimulation by 2-ketoisocaproate, there was a dissociation between an unaltered capacity of the 2-keto acid to decrease [^{45}Ca] outflow and a marked decrease of both the secondary rise in [^{45}Ca] outflow and release of insulin evoked by 2-ketoisocaproate. Moreover, in the presence of L-leucine and L-glutamine, the oxidation of which is unaffected [1] by CHX (1.0 mM), there was again a dissociation between an unaltered decrease in [^{45}Ca] outflow and a marked increase of both the secondary rise in effluent radioactivity and release of insulin. These findings clearly indicate that the CHX-induced perturbation of calcium fluxes is not solely attributable to changes in the metabolism of nutrient secretagogues. Instead, the present data suggest that CHX, like the inhibitor of glutathione reductase 1,2-bis(2-chloroethyl)-1-nitrosourea [12], may affect the intracellular distribution of Ca^{2+}, especially the process of Ca^{2+}-stimulated Ca^{2+} release from intracellular organelles thought to be responsible for the stimulant action of nutrient secretagogues upon [^{45}Ca] efflux from prelabelled islets [13]. In this respect, there was a close parallel between the effects of CHX upon the secondary rise in [^{45}Ca] outflow and secretion of insulin, respectively, both phenomena being inhibited by CHX in islets stimulated by either D-glucose or 2-ketoisocaproate and being enhanced by CHX in islets exposed to L-leucine and L-glutamine. In the latter case, our results confirm [1] that the secretory response is increased in islets pretreated with CHX (1.0 mM) prior to stimulation by L-leucine and L-glutamine, as was the case in our perifusion experiments, whereas it is either little affected or markedly decreased when the islets are immediately exposed to both CHX (1.0 and 2.0 mM, respectively) and the two amino acids (Table 6).

In addition to and independently of these changes in cationic fluxes, our data reveal that CHX abolished, in glucose-stimulated islets, the insulinotropic action of forskolin, theophylline and cytochalasin B. Even in islets stimulated by L-leucine and L-glutamine, and even under conditions in which the secretory response to these amino acids was unaffected by CHX, forskolin was virtually unable to augment insulin release. This is a most unusual situation. Indeed, whenever nutrient-stimulated insulin release is partially decreased, for instance as a result of either inhibition of nutrient utilization or decreased Ca^{2+} availability, the enhancing action of the above-mentioned insulinotropic agents remains unaffected. Usually, in order to abolish the secretory response to forskolin, theophylline or cytochalasin B, the control rate of nutrient-stimulated insulin release, as measured in the absence of these insulinotropic agents, needs to be decreased to the basal value [2, 3, 14]. Even in such a case, the facilitating action of these agents upon insulin release may occasionally still be disclosed [3, 14].

Our measurements of adenylate cyclase activity in islet homogenate and cyclic AMP production by intact islets indicate that the effect of CHX upon the secretory response to forskolin and theophylline cannot be ascribed to any major anomaly in the rate of cyclic AMP synthesis. The activity of protein kinase A was decreased in islets pretreated with CHX, but the enzymic responsiveness to cyclic AMP was preserved. Moreover, it seems unlikely that an impaired activity of the latter enzyme would account for the failure of cytochalasin B to augment glucose-stimulated insulin release in CHX-treated islets. Indeed, cytochalasin B is thought to facilitate insulin secretion by acting at the level of the contractile microfilamentous cell web and not through any primary effect on cyclic AMP generation [2]. An alternative explanation is suggested by the finding that CHX severely impaired the activity of transglutaminase which was recently proposed to participate in those motile events required for both the conversion of proinsulin and the release of insulin [15–17]. In the B-cells exposed to CHX, the virtual suppression of transglutaminase activity could thus prevent the supply of secretory granules to a readily releasable pool or, at least, impede their access to the exocytotic site. In considering this speculative proposal, it should be realized that the capacity to release insulin, or at least a modality for such a release, was not totally abolished in CHX-treated islets. Indeed, in such islets, the residual output of hormone evoked by D-glucose apparently still corresponded to an active process of secretion, itself dependent on both the metabolism of the hexose and the availability in extracellular Ca^{2+}.

In conclusion, the present data are compatible with the concept that CHX, in addition to any effect that it may exert upon nutrient catabolism and ionic fluxes, indeed alters the functional capacity of the effector system for insulin release in the pancreatic B-cell, possibly through inhibition of transglutaminase. Since the activity of the latter enzyme is modulated by its thiol/disulfide status [18], it is tempting to speculate, but remains to be proved, that the adverse effect of CHX upon the insulin-releasing effector system is secondary to a fall in GSH content of the islet cells.

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