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Chromium-induced Inhibition of Insulin Secretion from Isolated Islets of Langerhans

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Summary. $CrCl_3$, 0.25, 0.5, 1.0, and 1.5 mmol/l inhibited glucose-induced insulin secretion in a reversible and dose dependent manner. Cr also inhibited basal secretion of insulin in the presence of 5.5 mmol/l glucose and insulin secretion stimulated by 50 mmol/l K^+ or 15 mmol/l L-leucine. When 2 mmol/l theophylline was employed to potentiate the stimulatory effect of 16.5 mmol/l glucose, the inhibitory effect of 1.5 mmol/l Cr was reduced and that of 0.5 mmol/l Cr virtually abolished. A similar reduction in the inhibitory effect of Cr was observed when the medium calcium concentration was increased from 2.5 to 5, 7.5 and 12.5 mmol/l. Cr did not alter the conversion of ^{14}C-glucose to $^{14}CO_2$ or ^{45}Ca uptake by isolated islets. It is concluded that the inhibitory effect of Cr on insulin secretion may be mediated through interference with an intracellular function of Ca^{++} in the beta cell.

Key words: Insulin secretion, chromium, isolated rat islet, calcium interaction with chromium.

Chromium has been recognized as a trace element essential for both animal and human nutrition. It plays a role in the maintenance of normal glucose tolerance in at least some species of animals [1]. Rats fed a diet deficient in Cr developed impaired glucose tolerance, hyperglycaemia, and glycosuria [2]. Levine et al. [3] reported that Cr supplementation improved glucose tolerance in some elderly diabetic patients, but Sherman et al. [4] found in a younger diabetic population that prolonged Cr feeding failed to improve the glucose tolerance. Moreover, Cr treatment worsened the already existing hyperglycaemia in some diabetic patients [5]. Also, in experimental animals receiving a normally constituted diet, the administration of Cr was reported to increase plasma glucose levels [6, 7].

The biological effects of Cr on carbohydrate homeostasis, and its current clinical application in diabetes emphasizes the significance of defining the role of Cr in these processes. The purpose of the present study was to assess the effect of Cr on insulin secretion and to determine the mechanisms by which this trace element affects the secretory response of isolated pancreatic islets.

Materials and Methods

Isolated pancreatic islets were obtained by collagenase digestion [8] from adult (200–300 g) male Wistar rats allowed food and water ad libitum. All incubations were performed in a modified Krebs-Ringer bicarbonate medium (KRB) containing mmol/l: 115 NaCl, 5.0 KCl, 2.5 $CaCl_2$, 24 $NaHCO_3$, 1.0 $MgCl_2$ and 0.5% (w/v) bovine plasma albumin, Armour Pharmaceutical Co., Chicago, IL. The medium was equilibrated to pH 7.4 with a humidified mixture of O_2 (95%) and CO_2 (5%) and maintained at 37 °C.

D-glucose was obtained from the National Bureau of Standards, Washington, D. C.; L-Leucine from Sigma Chemical Co., St. Louis; Sucrose, KCl, $CrCl_3$, and toluene from Fisher Scientific Co., and theophylline from K + K Laboratories, Plainview, NJ; D-[U-^{14}C]glucose, [^{3}H]sucrose, and $^{45}CaCl_2$ from New England Nuclear Corp., Boston, MA.; Hydroxide of Hyamine-10X, PPO, and POPOP were obtained from Packard Instrument Co.

Chromium inhibition of insulin release was determined in a static incubation system. After isolation, pancreatic islets were transferred with the aid of a Pasteur pipette and dissection microscope to roundbottomed vials (11 mm diameter × 20 mm height) which contained 200 µl of KRB medium. Each vial contained 25 islets and 12–15 vials were employed per experiment. The glass incubation vials were inserted into scintillation vials equipped with rubber stoppers, gassed with O_2/CO_2 (95%/5%) and shaken in a Dubnoff Metabolic Shaker (70–100 cycles/min). The islets were then preincubated for 30 min in 200 µl of glucose-free medium containing different concentrations of $CrCl_3$ (0.25, 0.50, 1.0, and 1.5 mmol/l). The preincubation medium was removed and replaced with 200 µl of D-glucose (27.5 mmol/l) medium for

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Table 1. Effect of Cr on glucose-induced insulin release

	Ionic composition of medium		Insulin release μU/min per islet		% inhibition
	Cr^{+++}	Ca^{++}	Control	Experimental	
			Glucose 5.5 mmol/l		
1	0.25	2.5	0.59 ± 0.05 (15)	0.52 ± 0.06 (13)	12
2	0.50	2.5	0.53 ± 0.07 (8)	0.42 ± 0.04 (8)	25
3	1.0	2.5	0.54 ± 0.06 (10)	0.34 ± 0.03 (10)	38
4	1.5	2.5	0.59 ± 0.05 (15)	0.31 ± 0.02 (13)	47
			Glucose 27.5 mmol/l		
5	0.25	2.5	2.46 ± 0.12 (15)	2.06 ± 0.09 (15)	16
6	0.50	2.5	2.65 ± 0.08 (25)	1.84 ± 0.07 (30)	30
7	1.0	2.5	2.49 ± 0.11 (21)	1.62 ± 0.06 (26)	37
8	1.5	2.5	2.66 ± 0.09 (28)	1.36 ± 0.06 (31)	49
9	0	5.0		3.03 ± 0.18 (22)	
10	1.5	5.0		1.86 ± 0.09 (24)	38 10 vs 8 α 9 $P < 0.05$
11	0	7.5		2.97 ± 0.12 (27)	
12	1.5	7.5		2.04 ± 0.09 (28)	31 12 vs 8 α11 $P < 0.05$
13	0	12.5		2.79 ± 0.12 (22)	
14	1.5	12.5		1.82 ± 0.08 (24)	35 14 vs 8 α13 $P < 0.05$

After preincubation for 30 min in glucose-free KRB in the absence (control) or presence (experimental) of Cr, the medium was removed from the islets and replaced with medium containing glucose (5.5 or 27.5 mmol/l) and the indicated Cr concentration for a stimulation period of 30 min. Values represent the mean ± SEM with the number of observations in parenthesis. Statistical comparison with the corresponding control group showed significant differences $P < 0.01$–0.001) in all cases except 0.25 and 0.50 mm/l at 5.5 mmol/l glucose. The significances of differences among the groups with increasing calcium concentration were also estimated by factorial analysis of variance and the Newman-Keuls test

Table 2. Reversal of Cr inhibition of glucose-induced insulin release

Experimental condition	Insulin release (μU/min per islet)	
	First stimulation period	Second stimulation period
Control	2.64 ± 0.10 (13)	3.27 ± 0.17 (13)
Cr (0.5 mmol/l)	1.72 ± 0.09 (15)	3.12 ± 0.13 (15)
Cr (1.5 mmol/l)	1.35 ± 0.09 (15)	3.20 ± 0.18 (15)

Experimental procedures for the first stimulation period were as in Table 1. For the second stimulation period the islets were rinsed once with KRB medium (250 μl), and then incubated in KRB medium (200 μl) containing 5.5 mmol/l glucose for 15 min. This medium was then removed and replaced with incubation medium with 27.5 mmol/l glucose for a second stimulation period of 30 min. Values represent mean ± SEM with the number of observations in parenthesis

30 min in the presence of $CrCl_3$. Control groups were treated identically except in the absence of the test agent. At the end of the stimulation period the medium was removed and frozen for insulin assay by the method of Wright et al. [9] with crystalline porcine insulin as standard and ^{125}I-labelled porcine insulin as tracer. None of the experimental agents studied interfered with the measurement of insulin by the radioimmunoassay.

^{45}Ca Uptake

^{45}Ca uptake into isolated islets was measured with a double-isotope procedure described previously [10]. Twenty-five islets were preincubated for 30 min at 37 °C in 200 μl of glucose-free medium in the presence of 1.5 mmol/l Cr followed by incubation for 30 min in 200 μl medium containing 1.5 mmol/l Cr, 2.5 mmol/l $^{45}CaCl_2$ (0.22GBq/mmol), and 5 mmol/l [^{3}H]-sucrose (0.25GBq/mmol) as the extracellular marker, and 27.5 mmol/l glucose. ^{45}Ca uptake into the islets of the control group was studied in the absence of Cr.

Glucose Oxidation

Batches of 20–25 islets were preincubated for 30 min in glass incubation vials in glucose-free medium which contained 1.5 mmol/l Cr. At the end of the preincubation period, the medium was removed and 200 μl of incubation medium containing 1.5 mmol/l Cr and uniformly labelled 16 mmol/l [^{14}C]-D-glucose (0.022GBq mmol) was added. The measurement of $^{14}CO_2$ production was performed over 2 h as described previously [11].

Statistical Analysis

Insulin release is expressed as μU insulin/min per islet. In these experiments, each observation is based on 25 individual islets. Statistical analysis was made by the two tailed Student "t" test comparing the combined observations from separate experiments.

Results

Effect of Cr on Insulin Release Induced by Glucose and Other Agents

Table 1 summarizes the effect of Cr on glucose-induced insulin secretion by isolated rat islets. Chromium, 0.25–1.5 mmol/l, inhibited insulin release in a dose-dependent manner. Chromium, 1 and 1.5 mmol/l, significantly reduced basal secretion

Table 3. Effect of 1.5 mmol/l Cr on insulin secretion stimulated with K^+ or L-leucine

Experimental condition	Insulin release (μU/min per islet)		P value	% inhibition
	Control	Experimental		
K^+ (50 mmol/l)	2.87 ± 0.12 (16)	1.97 ± 0.12 (16)	<0.001	31
L-leucine (15 mmol/l)	2.92 ± 0.10 (21)	1.88 ± 0.09 (21)	<0.001	36

After preincubation for 30 min in KRB medium containing 5.5 mmol/l glucose in the absence (control) and presence (experimental) of Cr, the medium was removed from the islets and replaced with medium containing 5.5 mmol/l glucose and K^+ or L-leucine in the absence or presence of Cr for a stimulation period of 30 min. Values represent mean ± SEM with the number of observations in parenthesis

Table 4. Effect of Cr on insulin secretion stimulated with glucose and theophylline

Experimental condition	Insulin release (μU/min per islet)				
	Control	0.5 mmol/l Cr	% inhibition	1.5 mmol/l Cr	% inhibition
Glucose, 16.5 mmol/l	3.37 ± 0.30 (10)	2.12 ± 0.06 (10)	37	1.74 ± 0.12 (10)	49
Glucose, 16.5 mmol/l + Theophylline, 2 mmol/l	4.95 ± 0.27 (15)	4.37 ± 0.29 (15)	12	3.32 ± 0.19 (15)	33

Experimental procedures were identical to Table 1. Statistical comparison with corresponding control group showed significant differences ($P<0.005$–0.001) in all cases except for 0.5 mmol/l Cr and glucose + theophylline. Values represent the mean ± SEM with the number of observations in parenthesis

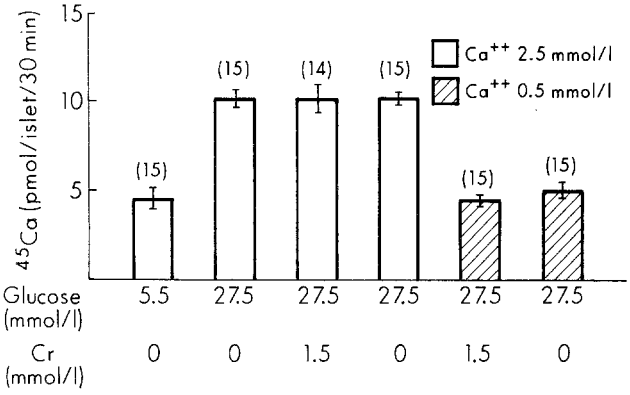

Fig. 1. Effect of glucose and Cr on ^{45}Ca uptake in isolated islets. Islets were preincubated for 30 min in KRB medium in the presence or absence of 1.5 mmol/l Cr. ^{45}Ca uptake of the islets was determined after incubation for 30 min in a medium containing ^{45}Ca, [^{3}H]sucrose and either 5.5 or 27.5 mmol/l glucose in the presence or absence of 1.5 mmol/l Cr. Calcium concentrations of medium were 2.5 mmol/l or 0.5 mmol/l as indicated. Values represent the mean ± SEM with the number of observations in parenthesis

at 5.5 mmol/l D-glucose. The inhibitory effect of Cr on glucose-induced insulin secretion was reversible (Table 2). Chromium, 1.5 mmol/l, also inhibited insulin secretion induced by 50 mmol/l potassium or 15 mmol/l L-leucine (Table 3).

Effect of Cr on Insulin Secretion Stimulated with Glucose and Theophylline

The addition of 2 mmol/l theophylline to medium containing (Table 4) 16.5 mmol/l glucose enhanced insulin release, compared with islets incubated without theophylline. In the presence of theophylline, the inhibitory effect of 1.5 mmol/l Cr was reduced from 49% to 33%. The inhibitory effect of 0.5 mmol/l Cr was completely prevented by theophylline.

Effects of Calcium on Cr Inhibition of Insulin Secretion

Increasing the medium calcium concentration from 2.5 to 5.0, 7.5 and 12.5 mmol/l increased the secretion of insulin by islets, but this increase was not statistically significant (Table 1). However, the inhibitory effect of Cr was significantly diminished in the presence of higher calcium concentrations in the medium (Table 1, lines 10, 12 and 14 vs 8).

Lack of Cr Effect on ^{45}Ca Uptake and D-[^{14}C]-Glucose Oxidation

^{45}Ca uptake was measured in islets in the presence of 5.5 and 27.5 mmol/l glucose in KRB medium containing 2.5 mmol/l calcium (Fig. 1). Increasing the concentration of glucose from 5.5 to 27.5 mmol/l enhanced ^{45}Ca uptake 2 fold (Fig. 1). However, 1.5 mmol/l Cr at a Ca^{++}/Cr^{+++} ratio of 1.67 did not alter glucose-induced ^{45}Ca uptake by isolated islets. When the calcium concentration in the KRB medium was reduced from 2.5 to 0.5 mmol/l, 1.5 mmol/l Cr at the Ca/Cr ratio of 0.33 also failed to modify glucose-induced ^{45}Ca uptake. Chromium at 1.5 mmol/l did not modify the rate of D-[^{14}C]-glucose (16.5 mmol/l) oxidation by isolated islets: control condition, 85.5 ±

2.6 (15 observations) vs Cr treatment, 83.8 ± 3.9 pmol D-glucose oxidized/2 h per islet.

Discussion

We have reported [7] that the acute administration of chromium to rats increases blood glucose levels and causes intolerance to a glucose load. This hyperglycaemic response to Cr is prompt in onset and lasts for only 5 h or less.

The present experiments were designed to evaluate the effect of Cr on insulin secretion from isolated rat islets and thus to elucidate the mechanism of Cr-induced hyperglycaemia. The experiments show that Cr inhibits insulin release from islets stimulated with glucose, leucine, or potassium in a dose-dependent manner. Cr also inhibits basal insulin secretion in the presence of 5.5 mmol/l glucose (a non-stimulatory concentration). The inhibition of non-stimulated insulin secretion suggests the existence of mechanisms for regulating the basal release of hormone.

The inhibitory effect of Cr on glucose-induced insulin secretion is completely reversible by removal of Cr from the incubation medium. Theophylline also totally reverses the inhibitory effect produced by a low concentration of Cr (0.5 mmol/l), and partially reverses the effect of a higher dose of Cr (1.5 mmol/l). This antagonistic effect of theophylline suggests a possible mechanism for the inhibitory effect of Cr on insulin secretion. One proposed mechanism by which theophylline potentiates glucose-induced insulin release is by increasing islet cell levels of cAMP which then leads to an intracellular translocation of calcium [12]. Thus, in the present study there may have been an interaction between Cr and Ca which resulted in inhibition of insulin secretion. This hypothesis was tested by increasing the medium calcium concentration from 2.5 to 5.0, 7.5, and 12.5 mmol/l. The higher calcium concentrations partially but significantly antagonized the inhibitory effect of Cr on insulin secretion.

The partial but significant antagonism of Cr-induced inhibition of insulin secretion by theophylline or increased calcium concentration, and the lack of an inhibitory effect of Cr on glucose metabolism and ^{45}Ca uptake support the hypothesis that Cr inhibits insulin release by an intracellular interaction with calcium. However, other mechanisms such as interaction with sulfhydryl groups of the plasma or granule membrane [13] cannot be ruled out.

The clinical use of Cr for improvement of glucose tolerance in diabetic patients is open to question in view of the effects in the rat, in which species Cr induces hyperglycaemia [7] as well as inhibition of insulin release from isolated islets. Although the continued administration (16–24 days) of Cr to rats failed to affect the response of animals to a glucose load [14], it has been reported that Cr treatment in some diabetic patients worsens the diabetes [5]. Therefore, Cr is not a drug for the general treatment of disturbances of carbohydrate metabolism. The apparent effect of supplementation with Cr can have useful practical importance only if Cr deficiency can be expected to exist in individual subjects.

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