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Effects of Thioacetamide on Pancreatic Islet B-Cell Function

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Thioacetamide (0.01–1.3 m*M*) fails to exert any significant immediate effect upon insulin release from rat isolated islets. However, when administered (4 µmol/g body wt) intraperitoneally 24 h before sacrifice, it reduced food intake and body weight and affected the secretory response of isolated islets to several secretagogues, despite unaltered insulin content of such islets. This coincided with a decrease in D-[U-^{14}C]glucose oxidation, total islet calcium content and the ionized calcium content of secretory granules in islet B-cells, and changes in both ^{133}Ba and ^{45}Ca net uptake. Likewise, in islets prepared from thioacetamide-injected rats and prelabeled with ^{45}Ca before perifusion, the cationic and insulin secretory responses to D-glucose or gliclazide, but not to the association of Ba^{2+} and theophylline in the absence of extracellular Ca^{2+}, often differed from that otherwise found in islets prepared from control rats. These findings are interpreted as indicative of an impaired capacity of Ca^{2+} sequestration by intracellular organelles in the islet B-cells of thioacetamide-treated rats.

Key Words: Thioacetamide; rat pancreatic islets; insulin release; ^{45}Ca and ^{131}Ba net uptake; D-[U-^{14}C]glucose oxidation; ^{45}Ca efflux from prelabeled islets.

Introduction

Thioacetamide has been used for several decades, to provoke *inter alia* hepatic damage, which is initiated by biotransformation of the hepatotoxic agent and is followed by oxidative stress, lipoperoxidation, alteration of cellular calcium handling, and, eventually, changes in DNA ploidy and distribution in the cell cycle phases *(1)*. Since 1991, when it became a specific entry in the *Index Medicus*, more than 120 articles were devoted to this agent. To our knowledge, however, the possible effects of thioacetamide on pancreatic islet B-cell function has so far not been investigated. Indeed, the sole consideration relative to both diabetes mellitus and thioacetamide concerns the enhanced hepatotoxicity and toxic outcome of thioacetamide in diabetes, e.g., in streptozotocin-induced diabetic rats *(2)*.

Because of the key role played by Ca^{2+} in the process of insulin release and in view of the already mentioned alteration of calcium handling caused by thioacetamide in hepatocytes, the aim of the present study was to explore possible changes in the functionality of insulin-producing islet B-cells in rats examined 24 h after a single intraperitoneal (ip) administration of thioacetamide in a dose of 4 mmol per kg body weight.

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Results

Effect of Thioacetamide on Food Intake, Body Weight and Other Metabolic Variables

The control and experimental animals used in this study had a comparable initial body weight (Table 1). Over the 24 h period preceding sacrifice, the control animals were given access to a limited amount of food (10 g/rat) and their body weight decreased by 7.4 ± 0.6 g/rat. The animals injected with thioacetamide (300 mg or 4 mmol/kg body weight) consumed somewhat less food than the control animals and lost significantly more weight over the 24 h separating the time of injection from that of sacrifice. The plasma glucose and plasma insulin concentrations, the insulin content of the islets, and the amount of secretory granules in the B-cells were not significantly different in control and thioacetamide-treated rats (Table 1).

Likewise, at the ultrastructural level, the density of insulin secretory granules appeared comparable in control and thioacetamide-treated rats (Fig. 1). As documented in Table 2, no significant difference was found between control and thioacetamide-treated animals in terms of the density of proinsulin and insulin immunoreactivity over B-cell secretory granules. As expected, the density of proinsulin immunoreactivity was higher ($p < 0.001$) over the pale granules located in the Golgi area (300 ± 30 particles/µm^2) than over the pale granules outside the Golgi area (110 ± 25 particles/µm^2), and further decreased ($p < 0.005$) over the dense secretory granules (17 ± 6 particles/µm^2; $n = 8$ in all cases). Inversely, the density of insulin immunoreactivity was lower ($p < 0.001$) over pale granules in the Golgi area (37 ± 6 particles/µm^2) than over pale granules outside the Golgi area

Table 1
Effect of Thioacetamide (4.0 mmol/kg Body Wt) Injected Intraperitoneally 24 h Prior to Sacrifice Upon Body Weight, Food Intake, and Other Metabolic Variables

	Control	Thioacetamide	*p*
Initial body weight (g)	215.6 ± 4.4 (68)	212.9 ± 3.1 (76)	N.S.
Change in body weight (g/24 h)	−7.4 ± 0.6 (68)	−12.4 ± 0.9 (76)	<0.001
Food intake (g/24 h)	10.0 ± 0.0	7.3 ± 0.6 (16)	<0.001
Plasma glucose (m*M*)	6.11 ± 0.39 (8)	6.88 ± 0.28 (12)	N.S.
Plasma insulin (μU/mL)	25.8 ± 4.3 (8)	21.4 ± 1.4 (12)	N.S.
Islet insulin content (mU/islet)	1.44 ± 0.08 (6)	1.62 ± 0.17 (9)	N.S.
Aldehyde-fuchsin staining[a]	0.494 ± 0.010 (12)	0.494 ± 0.006 (12)	N.S.

[a]Absorbance at 548 nm.

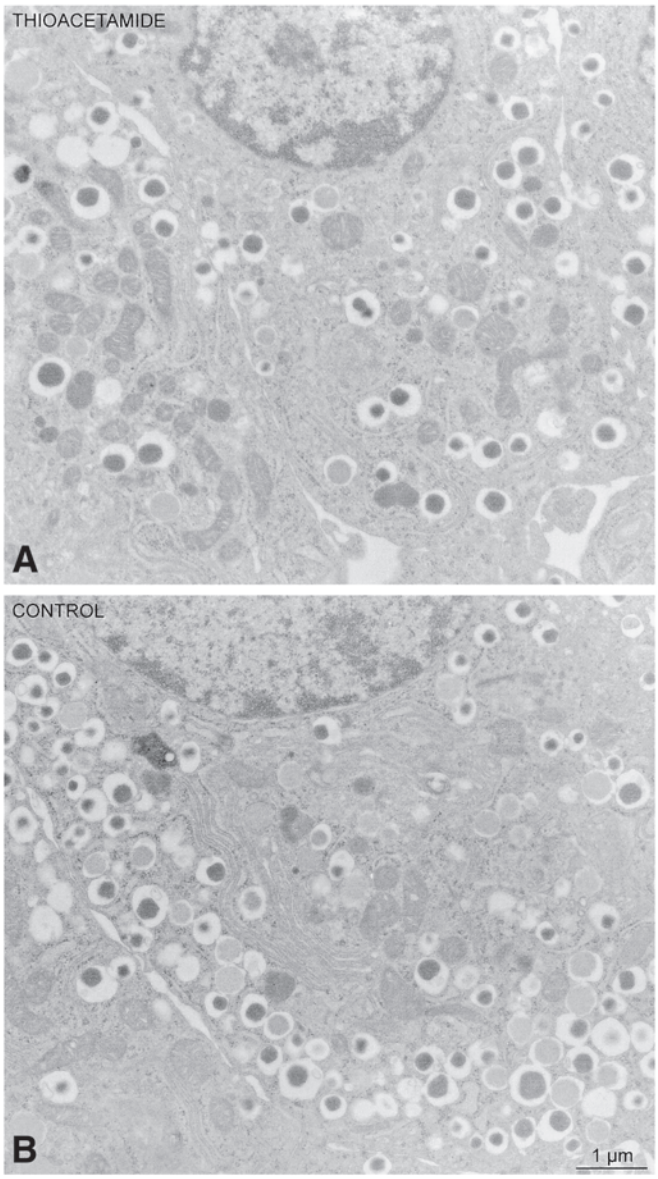

Fig. 1. Thin-section electron microscopy showing the comparable appearance of B-cell insulin secretory granules in the islets of Langerhans from thioacetamide-treated (**A**) and control (**B**) rats.

(111 ± 15 particles/μm^2), and further increased ($p < 0.001$) over the dense secretory granules (273 ± 27 particles/μm^2; $n = 9$ in all cases).

Insulin Release by Isolated Islets

Over a wide range of concentrations (0.013–1.33 m*M*), thioacetamide failed to exert any significant effect upon insulin release evoked by either D-glucose (8.3 m*M*) in the presence of Ca^{2+} (1.0 m*M*) or by the combination of Ba^{2+} (2.0 m*M*) and theophylline (1.4 m*M*) in the absence of Ca^{2+} in islets removed from fed rats not pretreated with thioacetamide (Table 3).

At normal Ca^{2+} concentration (1.0 m*M*), the basal release of insulin measured in the absence of glucose or at non-insulinotropic glucose concentrations (4.1 and 5.6 m*M*) was not significantly different in islets removed from control and thioacetamide-treated rats, respectively, the trend being toward a lower insulin output in the latter animals (Table 4). At an intermediate glucose concentration (8.3 m*M*), the secretory rate was much higher in the thioacetamide-injected rats. However, such a difference was no longer observed at higher glucose concentrations (11.1–27.8 m*M*). Likewise, at high glucose concentration (16.7 m*M*), the enhancing effect of either theophylline or cytochalasin B upon glucose-stimulated insulin output was virtually identical in control and thioacetamide-treated rats.

The release of insulin evoked by gliclazide (0.006–0.062 m*M*) in the presence of either a low (4.1 m*M*) or an intermediate (8.3 m*M*) concentration of glucose was much higher in the thioacetamide-treated rats than in the control animals. At the low concentration of glucose (4.1 m*M*), the gliclazide-induced increment in insulin output averaged 34.4 ± 3.9 and 53.8 ± 4.4 μU/90 min per islet in control and thioacetamide-injected rats, respectively, the difference between these two values being highly significant ($p < 0.005$). At the intermediate concentration of glucose (8.3 m*M*), the gliclazide-induced increment in insulin output averaged 195.4 ± 17.1 and 243.3 ± 16.7 μU/90 min per islet in control and thioacetamide-injected rats, respectively, the difference

Table 2
Density of Proinsulin and Insulin Immunoreactivity over B-Cell Secretory Granules

	Proinsulin		Insulin	
	Control	Thioacetamide	Control	Thioacetamide
Dense granules	7 ± 2 (4)[a]	26 ± 11 (4)	253 ± 44 (4)	289 ± 25 (5)
Pale granules in Golgi area	260 ± 49 (4)	339 ± 25 (4)	39 ± 6 (4)	36 ± 9 (5)
Pale granules outside Golgi area	76 ± 40 (4)	143 ± 24 (4)	109 ± 12 (4)	113 ± 27 (5)

[a]All results are expressed as number of gold particles per μm^2.

Table 3
Effect of Thioacetamide Upon Insulin Release Evoked by Either Glucose (in the Presence of Ca^{2+}) or the Combination of Ba^{2+} and Theophylline (in the Absence of Ca^{2+}) in Islets Removed from Fed Rats

	Glucose (8.3 m*M*) Ca^{2+} (1.0 m*M*)	No glucose No Ca^{2+} Ba^{2+} (2.0 m*M*) Theophylline (1.4 m*M*)
Control	93.5 ± 8.7 (9)[a]	84.3 ± 10.7 (9)
Thioacetamide (0.01 m*M*)	97.9 ± 10.5 (8)	92.4 ± 6.0 (8)
Thioacetamide (0.13 m*M*)	97.7 ± 8.9 (9)	82.4 ± 6.5 (9)
Thioacetamide (1.33 m*M*)	79.8 ± 8.6 (9)	72.8 ± 7.0 (9)

[a]Mean values (± SEM) are expressed as μU/90 min per islet.

Table 4
Effect of Glucose and Other Agents Upon Insulin Release by Islets Removed from Control and Thioacetamide-Injected Rats and Incubated at Normal Ca^{2+} Concentration (1.0 m*M*)

D-glucose (m*M*)	Other agent(s) (m*M*)	Insulin output (μU/90 min per islet)		
		Control	Thioacetamide	*p*
—	—	22.9 ± 5.7 (10)	12.0 ± 3.2 (10)	N.S.
4.1	—	21.0 ± 2.6 (9)	20.8 ± 2.6 (10)	N.S.
4.1.	Gliclazide (0.006–0.062)	55.4 ± 2.4 (19)	74.6 ± 2.8 (20)	<0.001
5.6	—	25.1 ± 4.1 (9)	15.6 ± 6.0 (10)	N.S.
5.6	No K^+	62.4 ± 4.3 (9)	29.4 ± 7.3 (10)	<0.005
5.6	No K^+ L + dantrolene Na (0.1)	34.0 ± 8.7 (10)	25.9 ± 5.5 (10)	N.S.
8.3	—	78.7 ± 12.3 (32)	152.2 ± 9.9 (33)	<0.001
8.3	Gliclazide (0.006–0.062)	274.1 ± 9.0 (20)	395.5 ± 14.0 (20)	<0.001
11.1	—	315.7 ± 21.9 (10)	328.0 ± 32.8 (10)	N.S.
16.7–27.8	—	475.6 ± 19.9 (31)	459.8 ± 19.3 (33)	N.S.
16.7	Theophylline (1.4)	834.1 ± 17.9 (10)	805.4 ± 38.5 (10)	N.S.
17.7	Cytochalasin B (0.02)	691.4 ± 22.3 (10)	691.5 ± 29.9 (10)	N.S.

between the two values being again significant ($p < 0.05$). However, the mean relative magnitude of the thioacetamide-induced increase in the response to gliclazide was higher at the low (+56.4 ± 17.1%) than at the intermediate (+24.5 ± 12.2%) concentration of glucose.

There was one situation in which the secretory response of control rats was higher than that of thioacetamide-treated animals. Thus, in the presence of 5.6 m*M* glucose, the omission of extracellular K^+ increased insulin output by 37.3 ± 5.9 μU/90 min per islet in control rats as distinct ($p < 0.05$) from only 13.8 ± 9.4 μU/90 min per islet in thioacetamide-treated animals, the latter increment failing to reach statistical significance. In the control rats, the release of insulin provoked by the omission of K^+ was significantly decreased in the presence of dantrolene sodium ($p < 0.02$).

Further experiments were performed in incubation media to which no or only little $CaCl_2$ (0.02–0.04 m*M*) had been added (Table 5). In such media, glucose (16.7 m*M*) or glicl-

Table 5
Effects of Glucose and Other Agents Upon Insulin Release by Islets Removed From Control and Thioacetamide-Injected Rats and Incubated in Media Containing No or Little $CaCl_2$

$CaCl_2$ (m*M*)	Glucose (m*M*)	Other agent(s) (m*M*)	Insulin output (μU/90 min per islet)		
			Control	Thioacetamide	*p*
—	—	—	19.1 ± 5.7 (10)	19.4 ± 8.1 (10)	N.S.
—	16.7	—	16.9 ± 3.2 (19)	32.0 ± 3.2 (18)	<0.005
0.02	16.7	—	11.8 ± 5.3 (9)	54.5 ± 7.1 (10)	<0.001
0.04	16.7	—	23.4 ± 3.5 (9)	92.8 ± 7.3 (10)	<0.001
—	—	Gliclazide (0.006)	20.0 ± 3.3 (26)	32.2 ± 6.8 (26)	N.S.
—	—	Ba^{2+} (2) + theophylline (1.4)	92.8 ± 6.2 (33)	161.7 ± 12.7 (33)	<0.001
—	—	Ba^{2+} (2) + theophylline (1.4) + suloctidil (0.005)	24.6 ± 11.7 (10)	25.9 ± 4.7 (10)	N.S.

Table 6
Metabolic and Ionic Variables in Islets Removed From Control and Thioacetamide-Injected Rats

Metabolic or ionic variables			Control	Thioacetamide	*p*
D-[U-^{14}C]glucose oxidation (pmol/120 min per islet)					
Glucose 8.3 m*M*	$CaCl_2$ 1.0 m*M*		21.8 ± 2.3 (10)	15.7 ± 0.5 (13)	<0.01
Glucose 16.7 m*M*	$CaCl_2$ 1.0 m*M*		32.8 ± 2.8 (11)	22.5 ± 1.3 (13)	<0.005
Glucose 16.7 m*M*	No $CaCl_2$		26.9 ± 5.4 (10)	23.8 ± 1.1 (13)	N.S.
^{133}Ba net uptake (pmol/60 min per islet)					
No glucose	No $CaCl_2$		16.8 ± 2.2 (22)	7.8 ± 0.4 (24)	<0.001
Glucose 8.3 m*M*	No $CaCl_2$		17.0 ± 2.5 (10)	9.7 ± 0.4 (12)	<0.005
^{45}Ca net uptake (pmol/90 min per islet)					
No glucose	$CaCl_2$ 0.06 m*M*		0.171 ± 0.006 (12)	0.201 ± 0.009 (12)	<0.02
Glucose 8.3 m*M*	$CaCl_2$ 0.06 m*M*		0.854 ± 0.042 (12)	0.836 ± 0.033 (12)	N.S.
Glucose 16.7 m*M*	$CaCl_2$ 0.06 m*M*		1.064 ± 0.049 (12)	1.164 ± 0.028 (12)	N.S.
No glucose	$CaCl_2$ 0.06 m*M*	Gliclazide (0.006 m*M*)	0.432 ± 0.015 (12)	0.499 ± 0.017 (12)	<0.01
No glucose	$CaCl_2$ 1.0 m*M*		1.37 ± 0.13 (12)	1.14 ± 0.07 (12)	N.S.
Glucose 8.3 m*M*	$CaCl_2$ 1.0 m*M*		2.34 ± 0.11 (21)	3.25 ± 0.14 (22)	<0.001
Glucose 16.7 m*M*	$CaCl_2$ 1.0 m*M*		3.42 ± 0.23 (20)	4.40 ± 0.20 (22)	<0.005
No glucose	$CaCl_2$ 1.0 m*M*	Gliclazide (0.006 m*M*)	2.22 ± 0.08 (12)	3.09 ± 0.13 (12)	<0.001
Glucose 8.3 m*M*	$CaCl_2$ 1.0 m*M*	Gliclazide (0.006 m*M*)	3.28 ± 0.16 (9)	3.89 ± 0.14 (10)	<0.02
Total islet Ca content (pmol/μg DNA)					
Glucose 2.5 m*M*	$CaCl_2$ 2.5 m*M*		699 ± 59 (12)	448 ± 25 (12)	<0.001
GBHA-Ca staining (absorbance at 496 nm)					
Glucose 2.5 m*M*	$CaCl_2$ 2.5 m*M*		0.131 ± 0.001 (12)	0.086 ± 0.001 (12)	<0.001

azide (0.006 m*M*) failed to stimulate insulin release above basal values in islets removed from control rats. However, in islets isolated from thioacetamide-injected rats, glucose significantly augmented insulin release, the magnitude of the secretory response being closely related to the Ca^{2+} concentration of the incubation medium in a range from the low Ca^{2+} value, possibly owing to contamination (in media receiving no added $CaCl_2$), to that found in the presence of 0.02 or 0.04 m*M* added $CaCl_2$. In the absence of added $CaCl_2$, gliclazide also slightly increased insulin release in the islets removed from thioacetamide-injected animals, but such an increase failed to achieve statistical significance. In the absence of added $CaCl_2$, the association of Ba^{2+} and theophylline markedly stimulated insulin release in both control and thioacetamide-treated rats, the secretory response being much higher in the latter than in the former case. Suloctidil suppressed the release of insulin caused by the association of Ba^{2+} and theophylline, in control as well as in thioacetamide-treated animals (Table 5).

Effect of Thioacetamide Upon Islet Metabolic and Ionic Variables

Islets removed from thioacetamide-injected rats oxidized D-[U-^{14}C]glucose at a lower rate than those obtained from control rats, whether at intermediate (8.3 m*M*) or high (16.7 m*M*) glucose concentration (Table 6). At the high glucose level, no significant difference in glucose oxidation between control and thioacetamide-treated rats was observed, however, when the islets were incubated in media deprived of $CaCl_2$.

The net uptake of ^{133}Ba by the islets was lower in thioacetamide-injected than in control rats, as measured in the absence of Ca^{2+} and either absence or presence of glucose (Table 6).

At a low concentration of $CaCl_2$ (0.06 m*M*), the basal net uptake of ^{45}Ca was slightly higher in thioacetamide-injected than in control rats (Table 6). However, in the presence of glucose (8.3 or 16.7 m*M*), no significant difference in ^{45}Ca net uptake was observed between the two groups of animals. Likewise, the gliclazide-induced increment in ^{45}Ca uptake was not significantly different in control (0.261 ± 0.016 pmol/90 min per islet) and thioacetamide-treated rats (0.298 ± 0.019 pmol/90 min per islet).

At normal $CaCl_2$ concentration (1.0 m*M*), the basal uptake of ^{45}Ca was not significantly affected by prior thioacetamide administration. However, in the presence of glucose (8.3 or 16.7 m*M*) and/or gliclazide (0.006 m*M*), the net uptake of ^{45}Ca was always higher in the thioacetamide-treated animals than in the control rats (Table 6).

The measurement of total Ca content and GBHA-Ca staining in islets from thioacetamide-treated rats provided results much lower than those collected in control animals (Table 6). The relative magnitude of the thioacetamide-induced decrease was similar for total Ca content (–36 ± 4%) and GBHA-Ca staining (–34 ± 1%).

Experiments in Perifused Islets

Further experiments were conducted in islets prelabeled with ^{45}Ca and placed in a perifusion chamber.

At normal extracellular Ca^{2+} concentration (1.0 m*M*), D-glucose (8.3 m*M*) provoked a biphasic change in ^{45}Ca outflow, consisting of a short-lived decrease in effluent radioactivity followed by a later and sustained increase in ^{45}Ca fractional outflow rate (Fig. 2). Likewise, when the administration of D-glucose was halted, a transient increase in ^{45}Ca efflux preceded the later fall in effluent radioactivity. Before introduction of D-glucose, the mean ^{45}Ca fractional outflow was lower, albeit not significantly so, in the islets from thioacetamide-treated rats than in those from control animals. There was also no significant difference between the two groups of rats in terms of the integrated increment in ^{45}Ca fractional outflow rate (min 45–68), difference in effluent radioactivity between the mean value recorded at min 40–44 and the paired nadir value reached shortly thereafter, or paired differences between such a nadir value and the peak value for ^{45}Ca outflow observed during the late period of exposure to D-glucose. Pooling the results obtained in control and thioacetamide-treated rats, these three variables averaged, respectively, 0.110 ± 0.027 × 10^{-2}/min, 0.379 ± 0.016 × 10^{-2}/min, and 0.551 ± 0.064 × 10^{-2}/min (n = 8 in all cases). The integrated increment in insulin output caused by D-glucose, above the paired basal value (min 40–44 and min 85–90), was lower ($p < 0.05$) in the thioacetamide-treated rats (30.6 ± 6.8 nU/min per islet; n = 4) than in the control animals (54.4 ± 5.4 nU/min per islet; n = 4). There was no significant difference ($p > 0.15$), however, between the absolute mean integrated value for insulin output during exposure to D-glucose (min 46 to 68), with an overall mean value of 185.7 ± 16.5 nU/min per islet (n = 8).

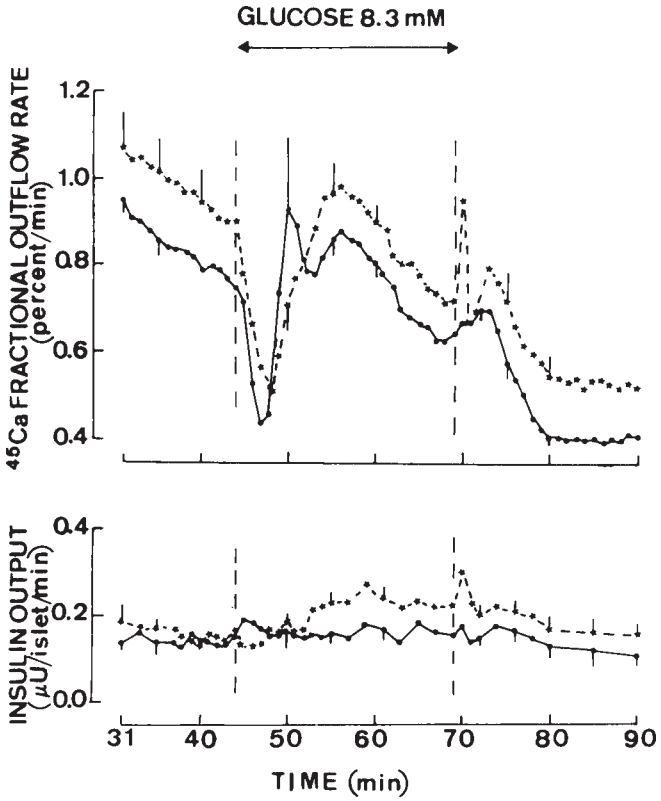

Fig. 2. ^{45}Ca fractional outflow rate and insulin release in islets obtained from control rats (open circles and dotted lines) or thioacetamide-injected animals (closed circles and solid line), perifused at normal Ca^{2+} concentration (1.0 m*M*), and exposed to a rise in D-glucose concentration from zero to 8.3 m*M* for 25 min (vertical dotted lines). Mean values (± SEM) refer to four individual experiments.

In the absence of D-glucose, gliclazide (62 μ*M*) caused a rapid, sustained, and rapidly reversible increase in ^{45}Ca outflow (Fig. 3). Both the peak and integrated values (min 45–60) for such an increment were slightly higher in the islets prepared from thioacetamide-treated rats rather than control animals, averaging, respectively, 132.4 ± 8.1 and 119.1 ± 5.2% (n = 4 in both cases) of the mean corresponding control values. Such differences only achieved statistical significance ($p < 0.02$), however, when pooling together the percentages of these two variables. The mean integrated increase in insulin output above basal value was not significantly different in the two groups of rats, with an overall mean value 387 ± 68 nU/min per islet (n = 8; $p < 0.001$). The peak value for insulin secretion recorded during exposure to gliclazide was significantly higher ($p < 0.01$), however, in the islets obtained from thioacetamide-treated rats as compared to control animals. In this set of experiments, like in the former one, the basal ^{45}Ca outflow rate (min 40–44) was lower, albeit

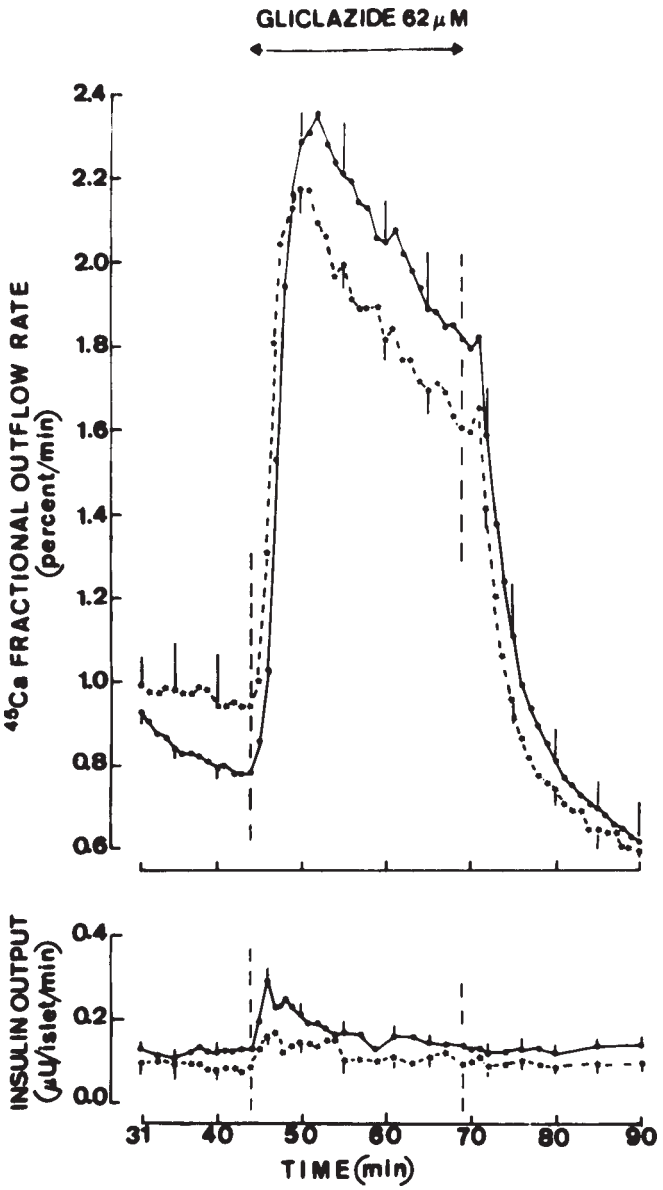

Fig. 3. ^{45}Ca fractional outflow rate and insulin release in islets obtained from control rats (open circles and dotted lines) or thioacetamide-injected animals (closed circles and solid line), perifused at normal Ca^{2+} concentration (1.0 m*M*) in the absence of D-glucose, and exposed to gliclazide (62 μ*M*) for 25 min (vertical dashed lines). Mean values (± SEM) refer to four individual experiments.

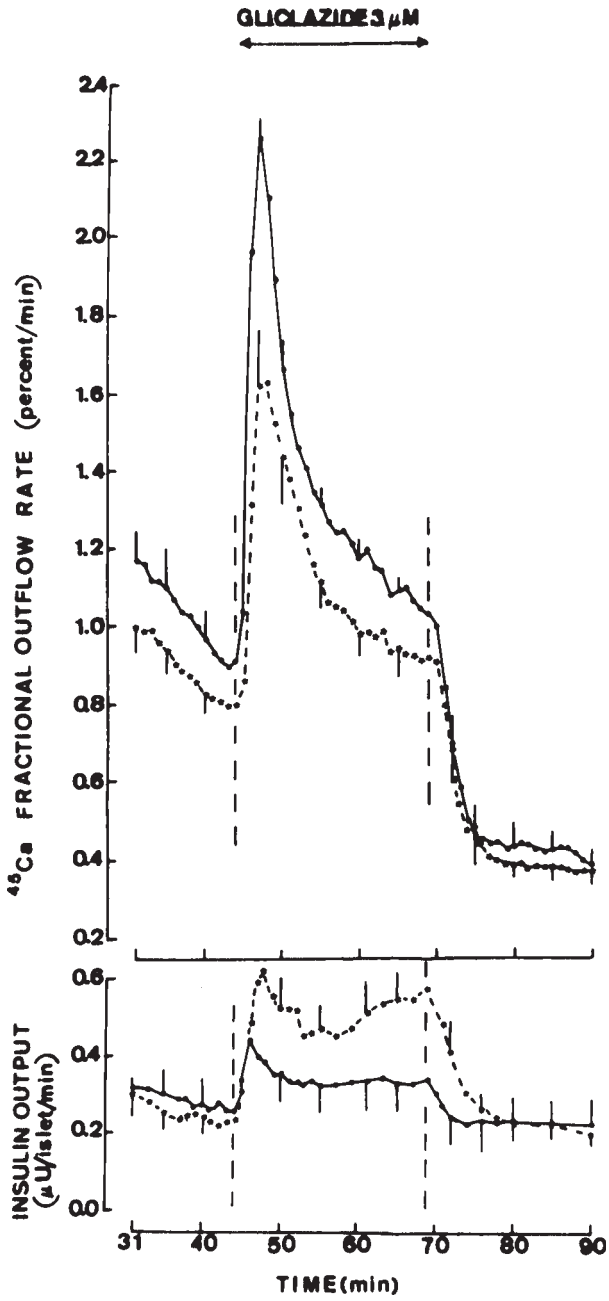

Fig. 4. ^{45}Ca fractional outflow rate and insulin release in islets obtained from control rats (open circles and dotted lines) or thioacetamide-injected animals (closed circles and solid line), perifused at normal Ca^{2+} concentration (1.0 m*M*) in the presence of D-glucose (8.3 m*M*), and exposed to gliclazide (3.1 μ*M*) for 25 min (vertical dashed lines). Mean values (± SEM) refer to four individual experiments.

not significantly so, in the thioacetamide-treated rats than in the control animals.

In the presence of 8.3 m*M* D-glucose, gliclazide (3.1 μ*M*) again caused a rapid, sustained, and rapidly reversible increase in ^{45}Ca outflow (Fig. 4). The mean basal ^{45}Ca outflow rate (min 40–44) was higher in the islets from thioacetamide-treated rats than in those from control animals. Such a difference failed, however, to achieve statistical significance. The peak value in effluent radioactivity, above the paired basal ^{45}Ca outflow (min 40–44), as well as the integrated increment in ^{45}Ca fractional outflow rate attributable to gliclazide (i.e., above the paired mean value recorded at min 40–44 and 86–90), were both significantly higher ($p < 0.02$ or less) in the thioacetamide-treated rats than in the control animals. Nevertheless, despite comparable values ($p > 0.6$ or more) for insulin output before introduction of gliclazide (min 40–44) and at the end of the experiments (min 86–90), both the peak value for insulin output above the paired mean value recorded between min 40 and 44 and gliclazide-induced integrated increment in insulin output

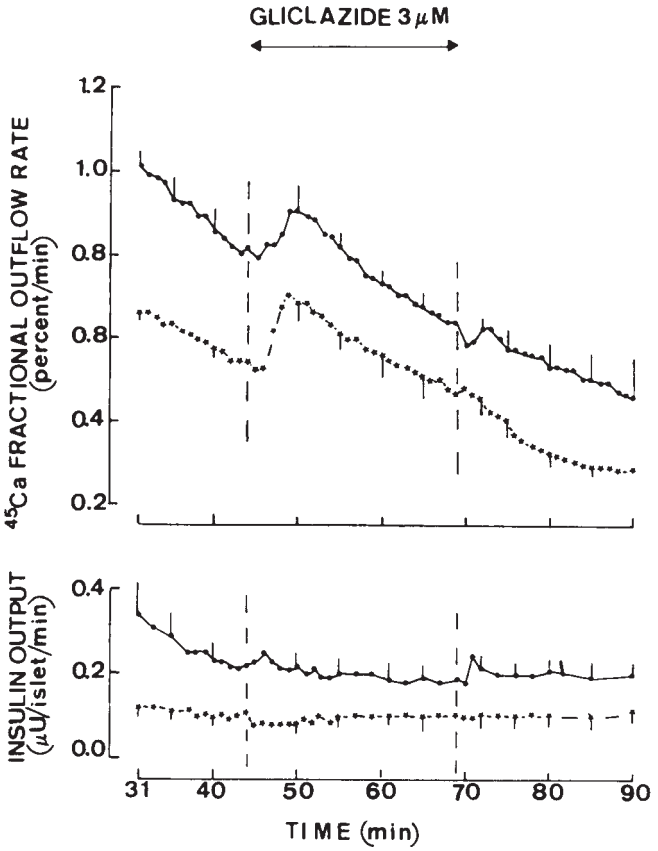

Fig. 5. ^{45}Ca fractional outflow rate and insulin release in islets obtained from control rats (open circles and dotted lines) or thioacetamide-injected animals (closed circles and solid line), perifused in the nominal absence of Ca^{2+} and presence of D-glucose (8.3 m*M*), and exposed to gliclazide (3.1 μ*M*) for 25 min (vertical dashed lines). Mean values (± SEM) refer to four individual experiments.

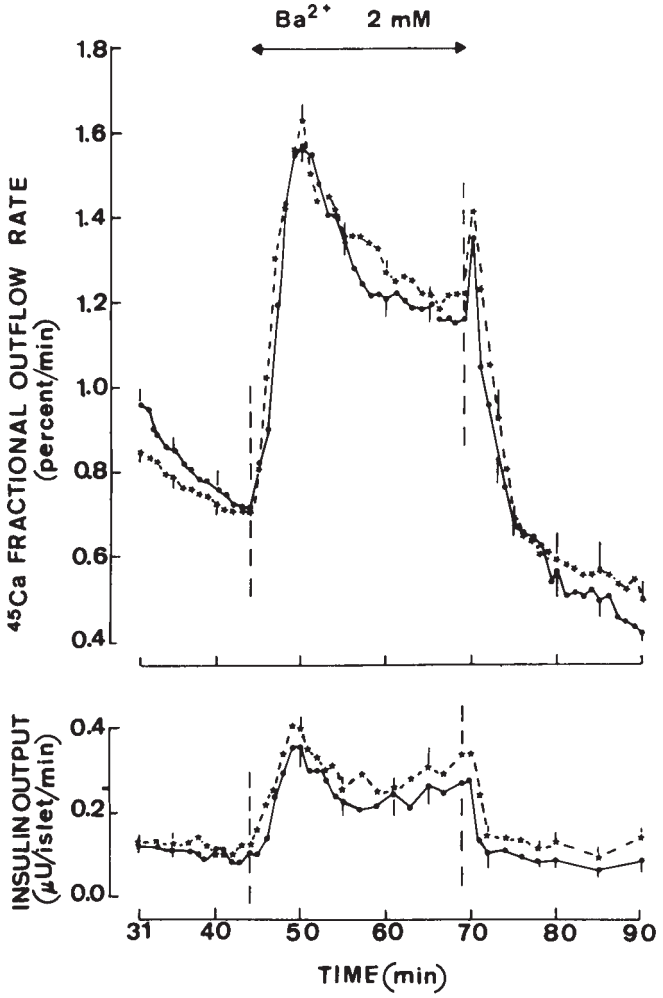

Fig. 6. ^{45}Ca fractional outflow rate and insulin release in islets obtained from control rats (open circles and dotted lines) or thioacetamide-injected animals (closed circles and solid line), perifused in the nominal absence of Ca^{2+} in the absence of D-glucose, and exposed to Ba^{2+} (2.0 m*M*) for 25 min (vertical dashed lines). Mean values (± SEM) refer to four individual experiments.

were significantly higher ($p < 0.05$) in control animals than in thioacetamide-treated rats.

When the same experiments were repeated in the absence of $CaCl_2$, the basal ^{45}Ca fractional outflow rate was again higher, albeit not significantly so, in the thioacetamide-treated rats than in the control animals (Fig. 5). Gliclazide again caused a rapid and sustained increase in ^{45}Ca outflow. The integrated increment in ^{45}Ca fractional outflow rate caused by gliclazide was comparable in control and thioacetamide-treated rats, with an overall mean value of $0.144 \pm 0.020 \times 10^{-2}$/min ($n = 8$), much lower ($p < 0.001$) than that found at normal extracellular Ca^{2+} concentration ($0.622 \pm 0.035 \times 10^{-2}$/min; $n = 8$). In the absence of $CaCl_2$, gliclazide failed to cause any sizeable increase in insulin output.

The comparison of experiments conducted at 8.3 m*M* D-glucose in the presence and absence of Ca^{2+} indicated that, in the latter case, (i) the mean basal ^{45}Ca FOR (min 40–44) represented only 78.0 ± 4.8% ($n = 8$; $p < 0.005$) of that found in the same type of rats in the presence of Ca^{2+} (100.0 ± 4.5%; $n = 8$); (ii) the integrated increment in ^{45}Ca FOR during exposure to gliclazide was dramatically lower, averaging no more than 23.9 ± 3.6% ($n = 8$; $p < 0.001$) of that found in the same type of rats at normal Ca^{2+} concentration (100.0 ± 3.2%; $n = 8$); (iii) the insulin output recorded before gliclazide administration (min 40–44) only represented 63.2 ± 12.0% ($n = 8$; $p < 0.06$) of that found in the same type of rats in the presence of Ca^{2+} (100.0 ± 12.7%; $n = 8$); and (iv) the gliclazide-induced increment in insulin output was abolished, while it averaged 196.3 ± 48.5 nU/min per islet ($n = 8$; $p < 0.005$) at normal extracellular Ca^{2+} concentration.

In the absence of D-glucose and $CaCl_2$, $BaCl_2$ (2 m*M*) provoked rapid, sustained, and rapidly reversible increases in both ^{45}Ca outflow and insulin output (Fig. 6). The magnitude of such increases was comparable in islets from control and thioacetamide-treated rats.

Discussion

The key findings made in the present study can be summarized as follows.

Thioacetamide treatment decreased food intake and accentuated the decrease in body weight otherwise seen in the control animals with restricted food supply, while failing to affect significantly plasma D-glucose and insulin concentrations. It also failed to affect islet insulin content, as judged by measurement of immunoreactive material, staining of secretory granules, or their abundance in sections examined by electron microscopy.

When tested in vitro, thioacetamide (0.013 to 1.33 m*M*) failed to affect significantly the secretory response to D-glucose (8.3 m*M*) or to the combination of Ba^{2+} (2.0 m*M*) and theophylline (1.4 m*M*). A different situation prevailed, however, in islets prepared from the thioacetamide-treated rats. Thus, in such a case, significant differences between thioacetamide-treated and control rats were often observed. They consisted, at normal Ca^{2+} concentration, in an increased secretory response to 8.3 m*M* D-glucose, but not higher concentrations of the hexose, and to gliclazide (both at 4.1 and 8.3 m*M* D-glucose). This contrasted with a lower secretory response to K^+ deprivation at 5.6 m*M* D-glucose in the islets of thioacetamide-treated rats as compared to control animals. At high concentration of D-glucose (16.7 m*M*), the enhancing action of theophylline and cytochalasin B displayed a comparable magnitude in thioacetamide-treated and control rats. Nevertheless, in the nominal absence of Ca^{2+} or at low concentrations of $CaCl_2$ (0.02 and 0.04 m*M*), the secretory response to 16.7 m*M* D-glucose was significantly higher in thioacetamide-treated rats than in control animals. Such was also the case for the secretory response to the association of Ba^{2+} and theophylline.

The metabolic and cationic data indicated impaired D-[U-^{14}C]glucose oxidation, at least at normal extracellular Ca^{2+} concentration (1.0 m*M*), decreased ^{133}Ba net uptake, lowered total islet Ca content, and, under most experimental conditions, increased $^{45}Ca^{2+}$ net uptake in the islets from thioacetamide-injected rats. The GBHA-Ca content of the B-cells was also decreased. GBHA stains the ionized Ca content of the secretory granules in B-cells. The granules contain about one third of the total islet Ca content *(3)*.

Last, the experiments conducted in perifused islets revealed a higher increment in ^{45}Ca fractional outflow rate in response to gliclazide administration, whether in the absence or presence of D-glucose and at normal extracellular Ca^{2+} concentration, but not so in the nominal absence of the divalent cation. At variance with the experiments conducted in freshly isolated and incubated islets, the insulinotropic action of D-glucose (8.3 m*M*) or gliclazide was not higher in perifused islets from thioacetamide-treated rats than in those from control animals. Likewise, the secretory response to Ba^{2+}, in the absence of Ca^{2+} and theophylline, was comparable in perifused islets from control and thioacetamide-injected rats. It should be stressed, however, that the perifused islets were first incubated for 60 min in the presence of D-glucose (16.7 m*M*) and Ca^{2+} (1.0 m*M*) and then perifused for 45 min before examination of their secretory and cationic response to selected insulinotropic agents.

The findings recorded in islets from control rats were in fair agreement with prior observations. This concerns, for instance, the effects upon insulin release, ^{45}Ca net uptake and ^{45}Ca efflux of D-glucose, gliclazide, K^+ deprivation, theophylline, cytochalasin B, low extracellular Ca^{2+} concentration, Ba^{2+}, and suloctidil *(4–10)*.

In our opinion, most, if not all, of the findings recorded in thioacetamide-treated rats point to a defect in the capacity of intracellular organelles to sequester Ca^{2+} in the islets. This proposal is indeed consistent with the following considerations. First, in hepatocytes prepared from rats that received a single administration of thioacetamide (6.6 mmol/kg body wt) 24 h before killing, the cytosolic Ca^{2+} concentration reaches a zenith value twice higher than the control value, and this coincides with a nadir value for the increase in such a concentration caused by 2,5-di(*tert*-butyl)-1,4-benzo hydroquinone *(11)*. Second, inhibition of respiratory metabolism with resulting increase in sodium content is observed in the liver of rats killed 24 h after thioacetamide administration (2.7 mmol/kg body wt) *(12)*. A comparable situation may prevail in the islets of thioacetamide-treated rats, as suggested by the impairment of D-[U-^{14}C]glucose oxidation and the lesser stimulation of insulin release caused by K^+ deprivation. Thus, the increase in insulin output caused by K^+ deprivation is currently ascribed to mobilization of Ca^{2+} from intracellular sites as a result of an increase in Na^+ islet content *(7,13)* and, as shown in the present study, is indeed opposed by sodium dantrolene. The latter redistribution of intracellular Ca^{2+} is likely to be impaired in the islets of thioacetamide-injected rats. Third, the increase in the net uptake of ^{45}Ca, as often observed in the islets from thioacetamide-treated rats, also points to alteration of the initial (time zero of incubation) cellular calcium homeostasis, as indeed documented by the measurements of total islet Ca content and GBHA-Ca staining. In this respect, the postulated perturbation of intracellular Ca^{2+} distribution may also account for the observed decrease in ^{133}Ba net uptake. Fourth, an increased lability of the intracellular organelle-bound Ca pool(s) could well be responsible for the higher secretory response of freshly isolated islets obtained from thioacetamide-injected rats to such secretagogues as D-glucose, gliclazide, and the association of Ba^{2+} and theophylline. In the former case, such an increased responsiveness was documented either at an intermediate concentration of D-glucose (8.3 m*M*) in islets exposed to a normal extracellular Ca^{2+} concentration or at a high concentration of the hexose (16.7 m*M*) in islets incubated in media containing little or no $CaCl_2$. It was not observed, however, at higher concentrations of D-glucose (11.1 to 27.8 m*M*) and normal Ca^{2+} concentration (1.0 m*M*). This again suggests that, under the latter conditions, the stimulation of oxidative metabolism may oppose the perturba-

tion of intracellular Ca^{2+} distribution, as could also be the case after preincubation of the islets under the same experimental conditions prior to the perifusion experiments (see above). Finally, even after such a preincubation, a residual increased lability of the intracellular organelle-bound Ca pool(s) was suggested by the higher increment in ^{45}Ca fractional outflow rate provoked by gliclazide at normal extracellular Ca^{2+} sequestration. In the case of the perifused islets exposed to Ba, however, a higher increment in ^{45}Ca efflux, as expected from the postulated perturbation of Ca^{2+} intracellular sequestration, was not observed, probably because of the restricted uptake of the former divalent cation.

In conclusion, insulin-producing pancreatic islet cells should now be added to the list of cell types affected by a prior administration of thioacetamide. Alteration of intracellular calcium homeostasis appears as a key determinant of the effects of thioacetamide in these islet cells. This is not meant to deny, however, that other factors may participate to the perturbation of B-cell function in the thioacetamide-injected rats.

Materials and Methods

Thioacetamide was purchased from Aldrich Chemical Co. (Milwaukee, WI).

All experiments were conducted in fed female albino rats. The control rats were given access to a limited amount of food (10 g/rat) for the last 24 h preceding sacrifice, while the experimental animals were injected intraperitoneally with thioacetamide (4 mmol/kg body wt) and then also given access to the same amount of food for the ensuing 24 h.

The methods used for the measurement of plasma D-glucose *(14)* and insulin *(15)* concentrations, insulin release by isolated pancreatic islets *(16)*, islet insulin content *(16)*, D-[U-^{14}C]glucose oxidation *(17)* and ^{133}Ba or ^{45}Ca net uptake *(4,9)* by incubated islets, and insulin output and ^{45}Ca fractional outflow rate from prelabeled and perifused islets *(18)* were previously described in the cited references.

Likewise, the procedures used for aldehyde-fuchsin staining of secretory granules *(19)*, total islet calcium content measurement, and glyoxal-*bis*-(2-hydroxyanil) (GBHA) staining of ionized calcium in pancreatic islet B-cells *(20–22)*, as well as for the examination of the islets by optic and electron microscopy *(7)*, were reported in prior publications. For the determination of proinsulin and insulin immunoreactive density over B-cell secretory granules, thin sections of Lowicryl-embedded islets were incubated with monoclonal antibodies to insulin and proinsulin and, then, with goat antimouse IgG conjugated with 10 nm gold particles *(23,24)*. The sections were stained and examined with the electron microscope. For each islet, 8–10 cells were photographed at a 21,000 magnification. The mean results obtained in 4–5 islets are given in Table 2.

All results are presented as mean values (± SEM) together with the number of individual determinations (in parentheses). The statistical significance of differences between mean values was assessed by use of Student's *t*-test.

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