Effect of Alloxan on Insulin and Glucagon Secretion in Perfused Isolated Islets

T. Tomita

Department of Pathology and Oncology, University of Kansas Medical Center, Kansas City, Kansas, USA

Summary. The effect of alloxan on insulin and glucagon secretion was investigated in perfused isolated rat islets. Five minutes of exposure to 1.4 mmol/l alloxan in a low-glucose medium (5.6 mmol/l) abolished subsequent leucine stimulated insulin and glucagon secretion. In a medium containing 19 mmol/l arginine and 3.3 mmol/l glucose, insulin secretion was only slightly diminished by alloxan pretreatment, whereas glucagon secretion was reduced to about 60% of controls. Exposure to alloxan in a high glucose medium (27.8 mmol/l) did not effect insulin or glucagon secretion.

Key words: Alloxan, isolated islets, perfusion, insulin, glucagon, leucine, arginine, glucose.

Alloxan inhibits glucose-induced insulin secretion in perfused isolated rat islets [1]. Inhibition can be prevented by including insulin-releasing concentrations of glucose in the medium; α -D-glucose gives more protection than β -D-glucose [2, 3]. Alloxan toxicity affects amino acid-stimulated insulin secretion as well [4].

The following effects of alloxan have been observed in isolated rat pancreas [5]: 1) absence of glucose inhibits the subsequent glucose-induced insulin secretion and abolishes the glucose-induced suppression on glucagon secretion. 2) Exposure to alloxan abolishes arginine-stimulated insulin secretion and produces complex effects on glucagon secretion.

In this communication additional evidence is presented which suggests that the glucose receptor system protects against the effect of alloxan on amino acid-stimulated hormone secretion in perfused isolated rat islets.

Materials and Methods

Isolation of Rat Islets

For each experiment, 200 islets were isolated from a male Sprague-Dawley rat (250–350 g) by the collagenase technique with minor modifications [6]. Briefly, 4–6 mg of collagenase type IV (Worthington) was added to 4 ml of a suspension of minced pancreatic tissue; the mixture was vigorously shaken for 8–10 min at 37 °C and then added to an equal volume of Hank's solution. After centrifuging, 4 ml of supernatant was discarded, and the precipitated islets were resuspended in the remainder, and again digested for 2–3 min with no additional collagenase. Each preparation yielded relatively large islets containing a rim of glucagon cells in the outer margin, as visualized in Epon embedded sections by electron microscopy [7].

Perfusion of Isolated Islets

The perfusion system was identical to that previously reported [1, 3]. Two chambers were perfused simultaneously: one without alloxan served as the control and the other with alloxan exposure as the experimental chamber. Perfusate from each chamber was collected at 1 min or 5 min intervals in graded tubes; immediately after collection 500 U of aprotinin (Sigma Chemical) was added per 1 ml of perfusate. Perfusate was collected at 5 min intervals during baseline secretion. Following exposure to alloxan, perfusate was collected at 1 min intervals for 10 min to detect the first phase of hormone secretion.

Insulin was determined by radioimmunoassay [8] with pork insulin (Eli Lilly) as the standard.

Glucagon Radioimmunoassay

Glucagon was radioimmunoassayed with beef-pork glucagon (Eli Lilly, Lot 258-V016-235) as the standard. After adding 0.1 ml of aprotinin (500 U/ml) and 0.1 ml of diluted rabbit anti-pork glucagon serum (YY 57 kindly supplied by Dr. K. Buchanan and Mr. R. Flanagan, N. Ireland) to each sample (0.2 ml), the tubes were incubated at 4 °C for 24 h. 0.1 ml of tracer [¹²⁵I]glucagon (New England Nuclear) and 0.1 ml of normal rabbit serum (1:100) as the carrier were then added, and the incubation was allowed to proceed for another 72 h. All of the added solutions were prepared in 0.05 mol/l phosphate buffer, pH 7.4, containing fraction V

Table 1. Effect of alloxan on insulin and glucagon secretion

	60 min perfusion (mmol/l)	5 min exposure		Insulin secretion		Glucagon secretion	
		glucose (mmol/l)	alloxan (mmol/l)	ng/100 islets/60 min Mean $\pm$ SE	Percent secretion	ng/100 islets/60 min Mean $\pm$ SE	Percent secretion
I. Leucine (30.5)	5.56	0 (5)*		345 $\pm$ 21	100%	11.19 $\pm$ 1.92	100%
	5.56	1.41		169 $\pm$ 29	49 $\pm$ 8 ^{a**}	6.23 $\pm$ 0.56	56 $\pm$ 5 ^c
II. Leucine (30.5)	27.8	0 (4)		350 $\pm$ 18	100%	9.85 $\pm$ 0.43	100%
	27.8	1.41		328 $\pm$ 18	94 $\pm$ 5 ^d	9.94 $\pm$ 1.33	101 $\pm$ 14 ^d
III. Arginine (19)– Glucose (3.33)	5.56	0 (7)		345 $\pm$ 19	100%	8.40 $\pm$ 0.74	100%
	5.56	1.41		327 $\pm$ 21	95 $\pm$ 6 ^d	5.41 $\pm$ 0.45	64 $\pm$ 5 ^b
IV. Arginine (19)– Glucose (3.33)	27.8	0 (5)		296 $\pm$ 28	100%	8.56 $\pm$ 1.07	100%
	27.8	1.41		303 $\pm$ 6	102 $\pm$ 2 ^d	7.31 $\pm$ 0.49	85 $\pm$ 6 ^d
V. Arginine (19)– Glucose (11.1)	5.56	0 (5)		440 $\pm$ 31	100%	8.02 $\pm$ 1.21	100%
	5.56	1.41		300 $\pm$ 40	68 $\pm$ 9 ^c	5.95 $\pm$ 0.87	74 $\pm$ 11 ^d
VI. Glucose (11.1)	5.56	0 (4)		436 $\pm$ 8	100%	3.65 $\pm$ 0.59	100%
	5.56	1.41		133 $\pm$ 9	31 $\pm$ 2 ^a	4.64 $\pm$ 0.50	127 $\pm$ 14 ^d
VII. Leucine (30.5)– Arginine (19)– Glucose (3.33)	5.56	0 (5)		436 $\pm$ 50	100%	9.22 $\pm$ 0.67	100%
	5.56	1.41		202 $\pm$ 30	46 $\pm$ 6 ^a	6.23 $\pm$ 0.47	68 $\pm$ 5 ^c

* Numbers in parentheses are the numbers of the experiments

** P values calculated by non-paired t-test

^a p < 0.001; ^b p < 0.01; ^c p < 0.05; ^d not significant **Fig. 1.** Test and control islets were perfused simultaneously with 5.6 mmol/l glucose for 50 min (control islets) or 45 min (test islets). The control islets (thin line) were subsequently perfused with 30.5 mmol/l leucine for 60 min while the test islets (thick line) were perfused with alloxan and 5.6 mmol/l glucose for 5 min, and then with 30.5 mmol/l leucine for 60 min. Vertical bars indicate standard errors. Upper: Insulin secretion (pg/islet/min) – Lower: Glucagon secretion (pg/islet/min) **Fig. 2.** Test and control islets were perfused with 5.6 mmol/l glucose for 45 min. The control islets (thin line) were subsequently perfused with 27.8 mmol/l glucose for 5 min and then with 30.5 mmol/l leucine for 60 min. The test islets (thick line) were perfused with alloxan and 27.8 mmol/l glucose for 5 min and then with 30.5 mmol/l leucine for 60 min. Upper: Insulin secretion (pg/islet/min) – Lower: Glucagon secretion (pg/islet/min)

Fig. 3. Test and control islets were perfused with 5.6 mmol/l glucose for 50 min (control) or 45 min (test). The control islets (thin line) were subsequently perfused with 19 mmol/l arginine-HCl plus 3.3 mmol/l glucose for 60 min. The test islets (thick line) were perfused with alloxan and 5.6 mmol/l glucose for 5 min and then with 19 mmol/l arginine-HCl plus 3.3 mmol/l glucose for 60 min. Upper: Insulin secretion (pg/islet/min) – Lower: Glucagon secretion (pg/islet/min)

Fig. 4. Test and control islets were perfused with a 5.6 mmol/l glucose for 50 min (control) or 45 min (test). The control islets (thin line) were subsequently perfused with 19 mmol/l arginine-HCl and 11.1 mmol/l glucose for 60 min. The test islets (thick line) were perfused with alloxan and 5.6 mmol/l glucose for 5 min and then with 19 mmol/l arginine-HCl and 11.1 mmol/l glucose for 60 min. Upper: Insulin secretion (pg/islet/min) – Lower: Glucagon secretion (pg/islet/min)

bovine albumin (1 g/l, Sigma Chemical). Separation of anti-serum bound from free hormone was accomplished by adding 1.2 ml of 99% isopropyl alcohol followed by centrifugation at 1000 g for 30 min at 4 °C. The pellets were counted with an automatic gamma system (Searle). The total binding of [125 I]glucagon after subtracting non-specific binding was 35–55%, and the sensitivity of the radioimmunoassay was 3.4 pg/0.2 ml.

Results

Effect of Alloxan on Leucine-stimulated Insulin and Glucagon Secretion

First phase insulin secretion occurred at about 5 min, and second phase at 15–30 min, after the change to leucine medium in control chambers. In contrast, after pretreatment with alloxan, insulin secretion was not significantly above the baseline (40 pg/min, islet). During a 60 min perfusion the total insulin secretion after alloxan pretreatment was about 50% of the control secretion (Table 1).

In case of glucagon secretion, two phases of secretion were more difficult to recognize than in the

case of insulin secretion (Fig. 1). After alloxan treatment the total glucagon secretion was about 60% of the control glucagon secretion (Table 1).

The effects of alloxan on leucine-stimulated hormone secretion were not observed when alloxan was added in combination with 27.8 mmol/l glucose (Fig. 2, Table 1).

Effect of Alloxan on Arginine-stimulated Insulin and Glucagon Secretion

A mixture of 19 mmol/l L-arginine-HCl and 3.3 mmol/l glucose induced both insulin and glucagon secretion. The insulin secretion occurred in two phases at about 5 min and at 20–30 min, respectively (Fig. 3). Alloxan pretreatment had no noticeable effect (Fig. 3, Table 1). In contrast, alloxan pretreatment diminished the total glucagon secretion to about 60% of the control level (Fig. 3, Table 1).

When alloxan was added in the presence of 27.8 mmol/l glucose, total insulin or glucagon secretion did not differ significantly between control and experimental chambers (Table 1).

Alloxan Inhibition of Arginine-Glucose (11.1 mmol/l) Stimulated Hormone Secretion

In medium containing 11.1 mmol/l glucose and 19 mmol/l arginine distinct first and second phases of insulin and glucagon secretion were observed (Fig. 4). The secretion of insulin, and perhaps also of glucagon, was inhibited by alloxan (Fig. 4, Table 1).

Alloxan Inhibition of Glucose-induced Insulin Secretion

Alloxan inhibited insulin release in response to 11.1 mmol/l glucose alone (Table 1). The glucagon release to 11.1 mmol/l glucose was not significantly influenced by alloxan (Table 1).

Alloxan Inhibition of Hormone Secretion in Response to Leucine and Arginine

In medium containing a combination of 30.5 mmol/l leucine, 19 mmol/l arginine and 3.3 mmol/l glucose insulin secretion was about the same as in medium containing 11 mmol/l glucose alone, or arginine plus 11 mmol/l glucose. Alloxan inhibited insulin secretion to less than one half of that of the controls (Table 1). The level of glucagon secretion was between that obtained by the leucine medium and that seen in the arginine plus glucose (3.33 mmol/l) medium. It was significantly inhibited by alloxan (Table 1).

Discussion

Dunn et al. [9] reported that alloxan damage to rat islets was confined to beta cells and that alpha cells were resistant. It is also known that alpha cells are the major surviving component in the islets of long term alloxan diabetic rats [10–12]. From previous [5] and present data, it appears that alloxan affects both beta and alpha cells, at least as regards function in the short-term perspective.

Leucine-stimulated insulin and glucagon secretion was markedly attenuated by alloxan, whereas in the presence of 3.3 mmol/l glucose the arginine-stimulated insulin secretion was not (Table 1). However, when insulin secretion was increased by adding 11.1 mmol/l glucose to the arginine medium, alloxan pretreatment decreased insulin secretion to the rate seen in response to arginine plus 3.3 mmol/l glucose. Similarly, insulin secretion induced by a mixture of leucine, arginine and 3.3 mmol/l glucose was reduced by alloxan pretreatment to the rate seen in response to arginine plus 3.3 mmol/l glucose.

Both insulin cells and glucagon cells appear to possess a glucose-receptor system [1, 13] by which glucose stimulates insulin secretion and suppresses glucagon secretion [13]. These receptor systems may somehow be involved in the glucose-induced protection against the effects of alloxan, not only on glucose-induced insulin release but also on leucine-induced insulin and glucagon secretion.

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References

1. Tomita T, Lacy PE, Matschinsky FM (1974) Effect of alloxan on insulin secretion in isolated rat islets perfused in vitro. *Diabetes* 23: 517–524
2. Niki A, Niki H, Miwa I (1976) Interaction of alloxan and anomers of D-glucose on glucose-induced insulin secretion and biosynthesis in vitro. *Diabetes* 25: 574–579
3. Tomita T, Kobayashi M (1976) Differential effects of alpha and beta-D-glucose on protection against alloxan toxicity in isolated islets. *Biochem Biophys Res Commun* 73: 791–798
4. Tomita T (1976) Effect of alloxan on arginine- and leucine-induced insulin secretion in isolated islets. *FEBS Lett* 72: 79–82
5. Goto F, Seino F, Imura H (1978) Modulation by alloxan of glucagon and insulin secretion in the isolated perfused rat pancreas. *Endocrinology* 102: 1496–1500
6. Lacy PE, Kostianovsky M (1969) Method for the isolation of intact islets of Langerhans from the rat pancreas. *Diabetes* 16: 35–39
7. Tomita T, Watanabe I (1976) The effect of alloxan on the permeability of isolated pancreatic islets to horseradish peroxidase. *Virchows Arch [Cell Pathol]* 22: 217–232
8. Makulu D, Vichick D, Wright PH (1969) Insulin immunoassay by back-titration using alcohol precipitation of insulin-antibody complexes. *Diabetes* 18: 660–669
9. Dunn JS, Duffy E, Gilmour MD (1944) Further observations on effects of alloxan on the pancreatic islets. *J Physiol (Lond)* 103: 233–243
10. Hoftiezer V, Carpenter AM (1973) Comparison of streptozotocin and alloxan-induced diabetes in the rat. *Diabetologia* 9: 178–189
11. Orci L (1976) The microanatomy of the islets of Langerhans. *Metabolism* 25 (Suppl): 1203–1313
12. Tomita T, Stranahan P (1976) Insulin secretion by isolated islets of alloxan diabetic islets. *Virchows Arch [Cell Pathol]* 20: 112–123
13. Pagliara AS, Stillings SN, Matschinsky FM (1977) Glucose and 3-O-methylglucose protection against alloxan poisoning of pancreatic alpha and beta cells. *Diabetes* 26: 973–979

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Tatsuo Tomita, M.D.
Department of Pathology
University of Kansas Medical Center
Kansas City, KS 66103
USA