Alloxan-Induced Alteration of Insulin Release, Rubidium Efflux and Glucose Metabolism in Rat Islets Stimulated by Various Secretagogues

J. C. Henquin, P. Malvaux, and A. E. Lambert

Unité de Diabète et Croissance, University of Louvain School of Medicine, UCL 54.74, Brussels, Belgium

Summary. Insulin release and ^{86}Rb efflux were studied in perfused rat islets exposed *in vitro* to alloxan (2 mmol/l) for 5 min. At a low glucose concentration, alloxan transiently increased ^{86}Rb efflux. Alloxan immediately and completely abolished the secretory response to glucose (15 mmol/l) and markedly delayed the reduction in ^{86}Rb efflux normally produced by the sugar. 3-*O*-methylglucose (20 mmol/l) provided complete protection against the alteration of ^{86}Rb efflux and partial protection against the inhibition of insulin release. Immediately after alloxan treatment, glyceraldehyde, α -ketoisocaproic acid and tolbutamide still induced a rapid release of insulin, but the late phase normally stimulated by glyceraldehyde and α -ketoisocaproic acid was inhibited. If islets were exposed to glyceraldehyde or tolbutamide 15 min after alloxan treatment, the rapid insulin release was also markedly impaired. Alloxan failed, however, to affect the ability of these three stimuli to reduce ^{86}Rb efflux from islet cells. Glucose oxidation and utilization were decreased in alloxan-treated islets and 3-*O*-methylglucose protected against this effect. The results show that the glucose recognition system in B-cells is the most rapidly and severely affected by alloxan. The drug also alters the response to other secretagogues, the insulin releasing properties of which can be impaired without alteration of their ability to reduce ^{86}Rb efflux.

Key words: Isolated rat islets, alloxan, perfusion, insulin release, rubidium efflux, glucose metabolism, glucose, glyceraldehyde, α -ketoisocaproic acid, tolbutamide.

pancreatic B-cells remain elusive. Elucidation of the molecular mechanisms of its action would certainly be of more than pharmacological interest. Indeed, observations that glucose can protect B-cells against alloxan toxicity [2, 3] make the drug a potential probe of the physiological stimulus-secretion coupling.

Present experimental evidence, recently discussed in detail [4], suggests that the B-cell membrane may be an important site of the action of alloxan. In mouse islets alloxan produces depolarization of B-cells [5] and inhibition of the univalent cation pump [6]. In rat islets it produces a loss of membrane-associated particles [7]. In toadfish islets [8], but not in rat islets [9], it increases the membrane permeability to mannitol, which is normally restricted to the extracellular space.

Recent studies have shown that glucose rapidly and markedly decreases potassium efflux from rat islet cells [10]. This supports the suggestion [11] that the depolarization of B-cells produced by the sugar [12–13] might be due to a reduction in K-permeability. The importance of these changes in K-permeability is underlined by the evidence that pharmacological agents which potentiate the effect of glucose on K-permeability also potentiate its insulin releasing effect, whereas those which augment K-permeability antagonize the insulinotropic action of the sugar [14–16].

In the present experiments, perfused isolated rat islets were exposed to alloxan for 5 min [17] in order to (a) compare the inhibitory effects of the drug on insulin release stimulated by various agents, (b) determine whether any alteration of the effect of these secretagogues is related to an impairment of their property to change the K-permeability of islet cells (evaluated by changes in ^{86}Rb efflux), and (c) investigate the effects of alloxan on glucose metabolism by islet cells.

Alloxan has been widely used to induce experimental diabetes [1], but the exact mechanisms by which the drug produces a relatively selective destruction of

Fig. 1. Effect of alloxan on ^{86}Rb efflux from rat islets perfused in the presence of 3 mmol/l glucose throughout. The stippled line shows the rate of ^{86}Rb efflux from control islets not treated with alloxan. As indicated by the arrows, alloxan (2 mmol/l) was added for 5 min (40–45 min ●—●) or 15 min (40–55 min ○—○). In the last-mentioned series of experiments, a new medium with freshly dissolved alloxan was used every 5 min. Values are means $\pm$ SEM of 4 experiments

Materials and Methods

Animals and Solutions

All experiments were performed with islets isolated from male Wistar rats (275–325 g) kept on a standard pellet diet and tap water ad libitum. They were killed 3 h after intraperitoneal injection of pilocarpine (20 mg/kg). The islets were obtained by combining mild collagenase digestion of the gland (5 mg collagenase per pancreas and shaking for 3.5 to 4 min) and microdissection of the partially digested pieces. Control experiments have shown that pretreatment of the animals with this dose of pilocarpine did not significantly alter the insulin response of the isolated islets to various secretagogues.

The medium utilized was Krebs-Ringer bicarbonate buffer [18], pH 7.4, gassed with O_2/CO_2 (94:6), supplemented with 0.5% (w/v) bovine serum albumin and containing 3 mmol/l glucose in basal conditions. For metabolic studies, 10 mmol/l HEPES (*N*-2-hydroxyethylpiperazine-*N*-2-ethanesulfonic acid) was added to the medium to prevent any change of pH in the small volumes of solutions used in these experiments. Because of rapid inactivation at 37 °C, alloxan (2 mmol/l) was dissolved in the appropriate pre-warmed medium immediately prior to use. This concentration of alloxan decreased the pH of the buffer by less than 0.2 units and a 5 min treatment with a medium acidified to 7.2 (by addition of HCl) did not affect the islet responses. Control experiments were also carried out with inactivated alloxan (3 h in solution at 37 °C before use).

Measurements of Insulin Release, Rubidium Efflux and Glucose Metabolism

The perfusion technique utilized to study insulin release and the method for measurement of immunoreactive insulin (IRI) have been previously reported [18].

The technique to monitor the efflux of ^{86}Rb from preloaded islets was similar to that recently described [10, 14]. Briefly, groups of 60 to 90 islets were first loaded with ^{86}Rb by incubation for 150 min in 0.5 ml basal medium supplemented with 0.2 mmol/l $^{86}\text{RbCl}$. They were then washed three times with 2 ml non-radioactive medium and placed in the perfusion chambers (0.3 ml), to which the perfusate, at 37 °C, was conveyed at a rate of 1.1 ml/min. ^{86}Rb in the effluent fractions (collected at 1 or 2 min intervals; 1500 to 15000 cpm per fraction) and remaining in the islets at the end of the experiment was counted by the Cerenkov radiation [10, 16]. For each collection interval, the fractional efflux of ^{86}Rb (^{86}Rb released during time interval/ ^{86}Rb remaining in tissue during that time interval) was calculated.

Glucose utilization by islet cells was measured by the production of [^3H]-water from D-[5- ^3H] glucose [19] and glucose oxidation by the conversion of D-[U- ^{14}C] glucose to [^{14}C]- CO_2 . Pools of islets were first preincubated at 37 °C in 10 ml medium containing glucose alone (3 mmol/l) or with 3-*O*-methylglucose (20 mmol/l). After 25 min, 5 ml of the same prewarmed medium with or without 6 mmol/l freshly dissolved alloxan was added; in the test groups the final concentration of alloxan was thus 2 mmol/l. Five min later, the islets were rapidly and extensively washed at room temperature before being transferred to the incubation tubes for 1 h at 37 °C in the following conditions: (a) utilization studies: 10 islets in 20 μl of medium containing 3 or 15 mmol/l D-[5- ^3H]-glucose (2.5 and 0.5 mCi/mmol, respectively). (b) oxidation studies: 10 islets in 50 μl of medium containing 3 or 15 mmol/l D-[U- ^{14}C]-glucose (6 and 1.2 mCi/mmol, respectively). Other technical aspects of these methods have been described elsewhere [18].

Chemicals

Alloxan monohydrate, 3-*O*-methyl-D-glucose and α -ketoisocaproic acid were obtained from Sigma Chemical Co., St. Louis, Mo., USA; HEPES was from British Drug Houses, Poole, Dorset, U. K.; D-glyceraldehyde was from Koch-Light Laboratories Ltd., Colnbrook Bucks, U. K.; collagenase (type IV; 200 U/mg) was from Worthington Biochemical Co., Freehold, N. J., USA; tolbutamide was supplied by Hoechst Research Laboratories, Frankfurt/Main, Germany. All other reagents were of analytical grade and purchased from Merck A. G., Darmstadt, Germany. The radioactive isotopes $^{86}\text{RbCl}$ (330–510 mCi/mmol), D-[U- ^{14}C] glucose (327 mCi/mmol) and D-[5- ^3H] glucose (1 Ci/mmol) were obtained from the Radiochemical Centre, Amersham, U. K.

Presentation of Results

All results are expressed as means $\pm$ SEM. The statistical significance of differences between experimental groups was assessed by Student's *t* test for unpaired data and the Wilcoxon rank-sum test.

Results

Effect of Alloxan at a Low Glucose Concentration

In control islets perfused with a medium containing 3 mmol/l glucose, the rate of ^{86}Rb efflux slowly decreased with time (Fig. 1). Exposure of the islets to 2 mmol/l alloxan for 5 min, at the same glucose concentration, had no significant effect on insulin release (not illustrated), but increased the rate of ^{86}Rb efflux. This increase in efflux rate was transient, even when

Fig. 2. Effect of alloxan on the reduction of ^{86}Rb efflux (upper panel) and the stimulation of insulin (IRI) release (lower panel) by glucose. Glucose concentration was increased from 3 to 15 mmol/l from 40 to 80 min. As indicated by the vertical arrows, alloxan (2 mmol/l) was added for 5 min (35–40 $\circ$ --- $\circ$). In this and all the following figures, the stippled line in the upper panel shows the rate of ^{86}Rb efflux from islets treated with alloxan between min 35 and 40 and maintained in 3 mmol/l glucose during the whole experiment. Values are means $\pm$ SEM of 4 experiments for ^{86}Rb efflux and 5 experiments for insulin release

alloxan remained present for 15 min (a freshly prepared solution was used every 5 min).

Effect of Alloxan on Glucose Stimulation

As shown in Fig. 2, stimulation with 15 mmol/l glucose produced a marked fall in the rate of ^{86}Rb efflux from control islets (upper panel) and a concomitant biphasic increase in insulin release (lower panel). Alloxan treatment for the last 5 min preceding glucose stimulation abolished insulin secretion (lower panel) and prevented glucose from rapidly decreasing the K-permeability in the islet cells. Thus, during the first 15 min of stimulation with 15 mmol/l glucose, the rate of ^{86}Rb efflux remained the same as in the presence of 3 mmol/l glucose; however, it finally reached the same low level as in control islets after 30 min of stimulation (Fig. 2, upper panel). Treatment of the islets with inactivated alloxan in the presence of 3 mmol/l glucose had no effect on ^{86}Rb

Fig. 3. 3-O-Methylglucose (3-O-MG) protection against alloxan alteration of the reduction of ^{86}Rb efflux (upper panel) and the stimulation of insulin (IRI) release (lower panel) by glucose. Glucose concentration was increased from 3 to 15 mmol/l from 40 to 80 min. 3-O-MG (20 mmol/l) was present 5 min before and during alloxan treatment. As indicated by the vertical arrows, alloxan (2 mmol/l) was added for 5 min (35–40). The broken lines in upper and lower panels show the control effect of 15 mmol/l glucose, without prior alloxan treatment. The stippled line in the upper panel as in Fig. 2. Values are means $\pm$ SEM of 4 experiments for ^{86}Rb efflux and 5 experiments for insulin release

efflux and did not alter the effect of a high glucose concentration (not shown).

Exposure of the islets *simultaneously* to 2 mmol/l alloxan (for 5 min) and 15 mmol/l glucose only resulted in a partial decrease in the insulin-releasing effect of the sugar. The first and second phases of insulin release were inhibited by 59 and 51% ($P < 0.005$, $n = 5$), respectively. In these conditions, ^{86}Rb efflux rate was not increased by alloxan but was reduced by high glucose, as in control islets (the fraction of ^{86}Rb lost per minute was 0.0170 ± 0.0005 after 5 min of 15 mmol/l glucose and 2 mmol/l alloxan together, as compared to 0.0166 ± 0.0003 after 5 min of 15 mmol/l glucose alone; $n = 3$).

In the experiments illustrated in Fig. 3, 3-O-methylglucose (20 mmol/l) was added to the perfusate 5 min before alloxan and was maintained during the 5 min of exposure to the drug. The presence of 3-O-methylglucose did not change basal insulin release, but provided partial protection against inhi-

Fig. 4. Effect of alloxan on the reduction of ⁸⁶Rb efflux (upper panel) and the stimulation of insulin (IRI) release (lower panel) by D-glyceraldehyde. Glucose concentration was 3 mmol/l throughout and D-glyceraldehyde (10 mmol/l) was present from 40 to 80 min. As indicated by the vertical arrows, alloxan (2 mmol/l) was added for 5 min (20–25 ▲·····▲) (35–40 ○---○). The stippled line in the upper panel as in Fig. 2. Values are means ± SEM of 5 control and 3 alloxan experiments for ⁸⁶Rb efflux and of 8 control and 5 alloxan experiments for insulin release

bition of the glucose effect by alloxan (Fig. 3, lower panel). The first and second phases of insulin secretion were inhibited by 57 and 32% respectively ($P < 0.01$ and $P < 0.02$, $n = 5$). 3-*O*-Methylglucose itself did not alter the rate of ⁸⁶Rb efflux from the islets, but prevented the increased efflux otherwise produced by alloxan. It also protected the ability of a high glucose concentration to reduce ⁸⁶Rb efflux rapidly (Fig. 3, upper panel). However, the changes in ⁸⁶Rb efflux and insulin release produced by 15 mmol/l glucose after exposure to alloxan together with 3-*O*-methylglucose were slightly delayed as compared to controls.

Effect of Alloxan on D-Glyceraldehyde Stimulation

In control islets, 10 mmol/l glyceraldehyde produced a rapid decrease in ⁸⁶Rb efflux and a simultaneous biphasic release of insulin (Fig. 4). Alloxan treatment

Fig. 5. Effect of alloxan on the reduction of ⁸⁶Rb efflux (upper panel) and the stimulation of insulin (IRI) release (lower panel) by α -ketoisocaproic acid (KIC). Glucose concentration was 3 mmol/l throughout and α -ketoisocaproic acid (10 mmol/l) was present from 40 to 80 min. As indicated by the vertical arrows, alloxan (2 mmol/l) was added for 5 min (35–40 ○---○). The stippled line in the upper panel as in Fig. 2. Values are means ± SEM of 3 experiments for ⁸⁶Rb efflux and 4 experiments for insulin release

during the last 5 min preceding stimulation delayed the early phase of release induced by the triose and markedly inhibited the late phase. If similar exposure to alloxan occurred earlier in the washing period and was followed by 15 min in basal medium, glyceraldehyde stimulation was almost ineffective (Fig. 4, lower panel, and Table 1). On the other hand, Fig. 4 (upper panel) shows that the rate of ⁸⁶Rb efflux, increased by alloxan, was quickly reduced upon addition of glyceraldehyde. This effect, although somewhat delayed as compared to controls, occurred much earlier than after stimulation with 15 mmol/l glucose (compare with Fig. 2) and was present even when the secretory response was almost suppressed.

The presence of 3-*O*-methylglucose during alloxan treatment completely protected the islets against inhibition of the insulinotropic action of glyceraldehyde (Table 1). The two phases of insulin release stimulated by glyceraldehyde were reduced by about 40% when no glucose was present in the medium and

were almost completely abolished if alloxan exposure occurred in these conditions (Table 1).

Effect of Alloxan on α -ketoisocaproic Acid Stimulation

Stimulation with α -ketoisocaproic acid (10 mmol/l) resulted in a concomitant decrease in the rate of ^{86}Rb efflux and a biphasic increase in insulin secretion (Fig. 5). Immediately after exposure of the islets to alloxan, α -ketoisocaproic acid still induced a slightly smaller rapid phase of insulin release (0.80 ± 0.05 ng/islet *vs* 1.08 ± 0.08 ng/islet in controls, $P < 0.05$, $n = 4$) followed by a markedly depressed late phase (lower panel) and remained able to reduce rapidly the rate of ^{86}Rb efflux (upper panel).

Effect of Alloxan on Tolbutamide Stimulation

At the glucose concentration used (3 mmol/l), tolbutamide (200 $\mu\text{g/ml}$) stimulated a monophasic release of insulin and decreased the rate of ^{86}Rb efflux (Fig. 6). Alloxan treatment of the islets immediately before stimulation did not significantly affect the releasing action of the sulphonylurea (1.04 ± 0.08 ng/islet, $n = 5$ *vs* 1.21 ± 0.05 ng/islet in controls, $n = 8$). By contrast, the insulin response to tolbutamide was markedly reduced (0.40 ± 0.05 ng/islet, $n = 5$, $P < 0.001$) if similar exposure to alloxan had occurred earlier in the washing period (lower panel). In addition, in a glucose-free medium, a 5 min treatment of the islets with alloxan inhibited the releasing effect of tolbutamide when tested 15 min later (0.17 ± 0.03 ng/islet *vs* 0.37 ± 0.06 ng/islet in controls, $P < 0.02$, $n = 5$). As shown in the upper panel of Fig. 6, the rapid reduction in ^{86}Rb

Fig. 6. Effect of alloxan on the reduction of ^{86}Rb efflux (upper panel) and the stimulation of insulin (IRI) release (lower panel) by tolbutamide. Glucose concentration was 3 mmol/l throughout and tolbutamide (200 $\mu\text{g/ml}$) was present from 40 to 80 min. As indicated by the vertical arrows, alloxan (2 mmol/l) was added for 5 min (20–25 $\blacktriangle$ ····· $\blacktriangle$) (35–40 $\circ$ --- $\circ$). The stippled line in the upper panel as in Fig. 2. Values are means $\pm$ SEM of 5 control and 3 alloxan experiments for ^{86}Rb efflux and of 8 control and 5 alloxan experiments for insulin release

Table 1. Effect of alloxan on the stimulation of insulin release by D-glyceraldehyde

Exp	n	Experimental conditions (mmol/l)			IRI release during stimulation (ng/islet)	
		Glucose	Alloxan	3-O-methylglucose	40.5–46 min	46–80 min
1	5	0	0	0	0.34 ± 0.03	6.70 ± 0.44
2	3	0	2	0	0.02 ± 0.00^a	0.35 ± 0.05^a
3	8	3	0	0	0.57 ± 0.04^b	11.11 ± 0.45^a
4	6	3	2	0	$0.10 \pm 0.02^{a,c}$	$1.36 \pm 0.35^{a,c}$
5	4	3	2	20	0.44 ± 0.06^d	10.46 ± 1.02^d

Immediately after isolation groups of 30 islets were transferred into the perfusion chambers for 80 min. Glyceraldehyde (10 mmol/l) was added to the medium from min 40 to 80. The glucose concentration indicated in the table remained the same throughout. Alloxan treatment lasted 5 min (min 20–25)

In experimental series 5, 3-O-methylglucose was present 5 min before and after as well as during alloxan treatment, thus from min 15 to 30. Values are means $\pm$ SEM of n experiments. Significance levels: ^a $P < 0.001$ *vs* Exp 1; ^b $P < 0.01$ *vs* Exp 1; ^c $P < 0.001$ *vs* Exp 3; ^d NS, $P > 0.05$ *vs* Exp 3

Table 2. Effect of alloxan on glucose metabolism by isolated islets

Experimental conditions during preincubation (mmol/l)	Glucose utilized (pmol/h/islet)	Glucose oxidized (pmol/h/islet)
<i>Glucose 3 mmol/l</i>		
Controls	35.0±0.9 (8)	11.1±0.9 (8)
Alloxan (2)	17.5±0.9 ^a (8)	5.6±0.7 ^a (8)
<i>Glucose 15 mmol/l</i>		
Controls	141.3±4.3 (11)	44.8±2.5 (11)
Alloxan (2)	64.7±2.7 ^a (11)	25.3±2.5 ^a (11)
Alloxan (2) + 3- <i>O</i> -methylglucose (20)	115.8±3.2 ^b (9)	42.6±2.1 (9)

Values are means ± SEM of the number of batches of islets given in parentheses. Significance levels: ^a $P < 0.001$ vs appropriate controls at the same glucose concentration; ^b $P < 0.001$ vs controls and alloxan alone, in the presence of 15 mmol/l glucose

efflux by tolbutamide persisted after alloxan treatment, even when secretion of insulin was inhibited.

Effect of Alloxan on Glucose Metabolism

In vitro treatment of the islets for 5 min with 2 mmol/l alloxan in the presence of 3 mmol/l glucose reduced glucose utilization and oxidation by roughly 50% both at a low (3 mmol/l) and a high (15 mmol/l) glucose concentration (Table 2). The presence of 3-*O*-methylglucose (20 mmol/l) during exposure to alloxan afforded partial protection of glucose utilization and complete protection of glucose oxidation.

Discussion

Effect of Alloxan on Insulin Release

In contrast to the well documented alloxan inhibition of glucose-stimulated insulin release, the effects of the drug on the response to other secretagogues have not been extensively studied. It has been reported that, after alloxan treatment *in vitro*, the rapid release of insulin stimulated by tolbutamide is preserved [17], whereas the response to leucine is partially inhibited [20]. The effects of alloxan on the stimulation by glyceraldehyde do not seem to have been investigated. It is known, however, that neither glyceraldehyde nor tolbutamide protects against the *in vivo* diabetogenic action of alloxan [21] nor against the *in vitro* alteration of glucose-stimulated insulin release [22] and proinsulin biosynthesis [23].

The present experiments demonstrate that the secretory response of B-cells to different stimuli is not uniformly impaired *immediately* after alloxan. As

previously reported [17] treatment of the islets with the drug during the 5 min immediately preceding stimulation is sufficient to abolish the response to glucose. By contrast, tolbutamide, glyceraldehyde and α -ketoisocaproic acid remain able to induce an almost normal rapid release after such treatment, the second phase of secretion stimulated by the two latter secretagogues being clearly reduced. Yet the differential effect of alloxan on the rapid and late phases of insulin release is only apparent. Thus, the early responses to tolbutamide and glyceraldehyde were also markedly inhibited if the stimulation was applied 15 min after alloxan treatment.

Since these experiments were carried out in the presence of 3 mmol/l glucose, the possibility was considered that the effect of the secretagogue itself was not markedly affected by alloxan, but that only the potentiation by the low glucose concentration was suppressed. Such a view is unlikely to explain the effects on α -ketoisocaproic acid stimulation, since the releasing properties of this leucine derivative are not augmented by a low glucose concentration [24]. Furthermore, it cannot account for the effect of alloxan on glyceraldehyde or tolbutamide stimulation; thus, the release of insulin induced by the triose in complete absence of glucose is also inhibited by alloxan and is much higher than that occurring after alloxan treatment in 3 mmol/l glucose (Table 2). Similarly, the insulin response to tolbutamide in a glucose-free medium is decreased by alloxan.

It appears that glucose stimulation of insulin release is by far the most sensitive to alloxan damage, but that the drug also produces a delayed inhibition of release when stimulated by agents structurally unrelated to glucose.

Effect of Alloxan on Rubidium Efflux

Because of its longer half-life, ⁸⁶Rb is a convenient substitute for ⁴²K, particularly when qualitative changes in the K-permeability are studied [10, 14, 16]. At a low glucose concentration, neither ⁴²K [10] nor ⁸⁶Rb efflux from the islet cells follows simple exponential kinetics (which would give a horizontal curve with the present mode of expression). The present experiments confirm that glucose [10, 11, 14] and tolbutamide [25] markedly reduce the potassium permeability in islet cells. They further demonstrate that the same effect is produced by glyceraldehyde and α -ketoisocaproic acid. This suggests that the four agents (provided α -ketoisocaproic acid has the same electrical properties as leucine) depolarize B cells [26, 27] by a similar mechanism.

By contrast, exposure of the islets to alloxan produced an increase in the rate of ⁸⁶Rb efflux prevented by a high concentration of 3-*O*-methylglucose or glu-

cose. Such an effect of alloxan was not found in islets of ob/ob mice [6], probably because measurements of the amount of ^{86}Rb retained in the tissue are not sensitive enough to detect a transient increase in ^{86}Rb efflux. Although this increase in K-permeability could conceivably be the result of damage to the cell membrane, several arguments do not support this possibility: (a) the effect is transient even when alloxan is present for 15 min; (b) it is not accompanied by a leak of insulin in our system; (c) a similar dose of alloxan does not augment the permeability of rat islet cell membranes to sucrose [9]; and (d) it is quickly reversed by glyceraldehyde, tolbutamide and α -ketoisocaproic acid. We would suggest rather that the blockade [6] of an electrogenic Na/K pump [27] is, at least partially, the cause of the depolarization measured in alloxan treated B-cells [5] and that the increase in ^{86}Rb efflux is the consequence of this fall in membrane potential. The possible interrelationships between ionic, electrical and metabolic changes produced by alloxan are difficult to evaluate. For instance, an arrest of the Na/K pump can theoretically be the cause as well as the consequence of decreased metabolism. However, the depolarization of B-cells produced by alloxan is not expected to result from an impaired energy production, since classical uncouplers or inhibitors of glucose metabolism hyperpolarize B-cells [12, 28]. A direct effect of alloxan on the Na/K pump is thus plausible [6].

Similar to its insulin releasing properties, the ability of glucose to reduce K-permeability in islet cells appears exquisitely sensitive to alloxan. Thus, among the four agents tested, only glucose was unable to reduce rapidly ^{86}Rb efflux from islets initially exposed to alloxan. Since normal glucose metabolism is required for glucose to decrease K-permeability [10], alloxan-induced metabolic alterations might be involved. In several experimental conditions alloxan barely affected the ability of secretagogues to reduce K-permeability in islet cells, whereas it markedly altered their insulin releasing effect. Such a dissociation has also been found in the absence of extracellular calcium [10] and suggests that the alloxan block of insulin release is not at the earliest steps of stimulus-secretion coupling. However, a complete understanding of the mechanisms underlying secretagogue-induced changes in K-permeability is obviously needed to propose a rational explanation of the present observations.

Effect of Alloxan on Glucose Metabolism

The present results clearly show that a brief alloxan treatment *in vitro* severely alters both glucose utilization ("glycolysis") and oxidation in islet cells. 3-*O*-Methylglucose protects against this deleterious

effect, which cannot be accounted for by a reduced glucose transport in islet cells [9]. An impairment of glucose oxidation by mouse islets has also been reported after *in vivo* treatment of the animals with alloxan [29] or *in vitro* exposure of the islets to the drug [30]. It is clear, however, that detailed time course studies are necessary to establish whether there is a causal relationship between the alteration of glucose metabolism and the inhibition of insulin release. Nevertheless, these observations and the evidence of a strong inhibition of proinsulin biosynthesis in isolated islets after *in vivo* [31] or *in vitro* [23, 32] treatment by alloxan show that, besides its membrane effects, alloxan also has intracellular effects.

Mechanism of Alloxan Action

Although it remains difficult to propose a complete model of the effects of alloxan, the following suggestions can be made. The recognition of glucose by B-cells is more rapidly and more severely affected by alloxan than the recognition of other secretagogues. However, after some delay, their releasing effect is markedly impaired, without B-cells being totally unresponsive, since the decrease in K-permeability is only marginally affected. It does not appear possible to explain these observations by an action of alloxan restricted to a putative membrane glucoreceptor. The possibility should be considered that alloxan enters B-cells [33, 34] via the hexose carrier system where antagonists of glucose transport (3-*O*-methylglucose, cytochalasin B, phlorizin) exert their protective action [4, 17, 21, 34–36]. Alloxan would then interfere with cellular events crucial for the response to glucose and subsequently lead to a more general dysfunctioning of the releasing machinery.

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Dr. J. C. Henquin
Unité de Diabète et Croissance
University of Louvain, UCL 54.74
B-1200 Brussels
Belgium