Alloxan Stimulation and Inhibition of Insulin Release from Isolated Rat Islets of Langerhans

Daniel C. Weaver, Ph.D., Michael L. McDaniel, Ph.D., Stephen P. Naber, Ph.D., C. David Barry, Ph.D., and Paul E. Lacy, M.D., Ph.D., St. Louis

SUMMARY

The rate of alloxan-induced insulin release was measured from rat islets maintained in a simple perifusion system. Insulin release during the five-minute exposure to alloxan reached its maximum rate after two to three minutes of the exposure and then rapidly declined. This insulin release was dependent upon extracellular calcium and was associated with an increased ^{45}Ca uptake by isolated islets. Once exposed to alloxan, however, the islets did not release insulin when stimulated again with D-glucose or alloxan. These effects of alloxan on insulin release (stimulation and subsequent inhibition) and the increased ^{45}Ca uptake were prevented by the presence of 3-0-methyl-D-glucose during the alloxan exposure. These findings indicate a close correlation between alloxan-induced insulin release and the subsequent inhibition of further insulin release.

D-glucose, when present during the entire five-minute exposure to alloxan, protected competitively against alloxan inhibition of insulin release. In addition, D-glucose, when present immediately after brief (one to three minutes) alloxan exposures, reversed some of the subsequent inhibition of insulin release. These findings suggest that alloxan and D-glucose were competing for a common site on the β-cell. The possibility of this site being a receptor responsible for the initiation of insulin release is discussed.

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Previous studies have shown that a five-minute exposure of isolated rat islets to alloxan inhibits subsequent glucose-induced insulin release.[1] In turn, the presence of high concentrations of D-glucose during the alloxan exposure protects the islets against subsequent inhibition of insulin release. The protection by glucose is stereospecific for the D-isomer and shows preference for the alpha anomer of D-glucose at low concentrations.[2] In addition, 3-0-methyl-D-glucose, a nonmetabolized glucose analog,[3] protects against alloxan inhibition of insulin release.[1] These findings suggest that D-glucose and alloxan may interact at a site where insulin is released.

Recently, a brief burst of insulin release was detected during the five-minute exposure to alloxan in isolated islets[4] and in isolated perfused rat pancreas.[5] To examine quantitatively this alloxan-induced insulin release and its relationship to the subsequent inhibition of further insulin release, a modified perifusion system was developed with a total lag time of less than 10 seconds. This perifusion system and a simple static incubation system developed previously allowed for the determination of the precise dynamics of alloxan's effects on insulin release. The purpose of the present study is to use alloxan as a probe to examine the molecular mechanisms of insulin secretion by determining the dynamics of alloxan-induced insulin release, the rate of alloxan inhibition of insulin release,

From the Department of Pathology, the Computer Systems Laboratory, and the Department of Physiology and Biophysics, Washington University School of Medicine, St. Louis, Missouri 63110.

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and the interactions of D-glucose and alloxan on the insulin release mechanism.

MATERIALS AND METHODS

Medium and chemicals. Incubations, unless indicated otherwise, were accomplished with a modified Krebs-Ringer bicarbonate medium containing 115 mM NaCl, 24 mM $NaHCO_3$, 5.0 mM KCl, 1 mM $MgCl_2$, 2.5 mM $CaCl_2$, albumin (0.5 per cent w/v, bovine plasma albumin, Armour Pharmaceutical Company, Chicago, Illinois) and, as required, D-glucose (dextrose, National Bureau of Standards, Washington, D.C.), [^{3}H]sucrose, $^{45}CaCl_2$ (New England Nuclear, Boston, Massachusetts), alloxan monohydrate, ethyleneglycol-bis-(β-aminoethyl ether) N,N′-tetraacetic acid (EGTA), and 3-0-methyl-D-glucose (Sigma Chemical Co., St. Louis, Missouri). The medium was equilibrated for at least 30 minutes to 37° C. and pH 7.4 with a humidified mixture of O_2/CO_2 (95 per cent/5 per cent) to allow complete mutarotation of the anomers of D-glucose before the islet incubation.

Calcium-free medium was made by omitting $CaCl_2$ from the Krebs-Ringer bicarbonate medium and adding 1 mM EGTA (neutralized to pH 7.4).

Alloxan assay. A stock solution of alloxan was prepared in 1 mM HCl, and the concentration was determined spectrophotometrically at 270 nm ($\epsilon = 980$ $M^{-1} \cdot cm.^{-1}$) in 50 mM phosphate buffer (pH 7.4) by the method of Patterson et al.[6] Since alloxan decomposes rapidly at pH 7.4 and 37° C.,[7,8] a portion of the stock solution was added to previously warmed and gassed medium within 10 seconds of use. No chemical reaction could be detected between alloxan and D-glucose by spectrophotometric,[6] fluorometric,[9] or thin-layer chromatographic[10] methods of analysis.

Perifusion technic. The perifusion apparatus was modified from that described previously[11] to reduce the total lag time by decreasing its internal volume. Figure 1 illustrates that the perifusion chamber (13 mm., Millipore, Bedford, Massachusetts) was altered to reduce the lag time on both the afferent and efferent sides of the chamber. The afferent opening was enlarged so that the islets received immediate contact with the surrounding medium. On the efferent side, the internal volume of the chamber was reduced by first inserting plastic tubing (I.D. = 0.015 inches, Clay-Adams, Parsippany, New Jersey) into the efferent opening and then filling the remainder of that side of the chamber with paraffin. The plastic tubing (20 cm.) from the efferent side of the chamber was connected to a short segment (9 cm.) of Silastic tubing (I.D. = 0.030 inches, Dow-Corning, Midland, Michigan) contained within the peristaltic pump (Harvard Apparatus Co., Millis, Massachusetts). The perifusion chamber was placed directly into a reservoir of medium (75 to 100 ml.) in the water bath at 37° C. The medium was drawn by the peristaltic action of the pump through the perifusion chamber at a flow rate of 0.7 to 0.9 ml. per minute. These modifications reduced the total lag in the perifusion apparatus from about five minutes to less than 10 seconds, as was determined with ^{125}I-labeled insulin.

Islets were isolated from the pancreases of male Wistar rats (200 to 300 gm.) by the collagenase technic[11,12] and then transferred to the perifusion chamber in the following way: A 5-μ Millipore filter (Gelman, Ann Arbor, Michigan) and gasket were placed on the surface of the plastic mesh in the chamber. The afferent part of the chamber was then screwed tightly in place and the chamber was filled with medium. Islets (about 150) were then dripped onto the surface of the Millipore filter with a

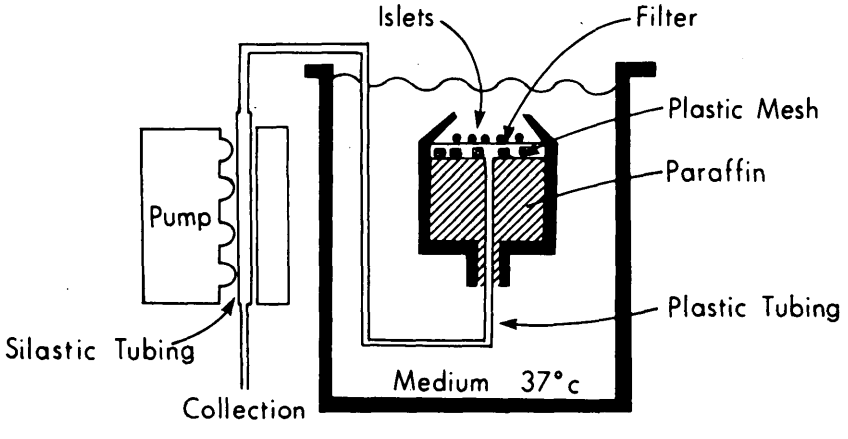

FIGURE 1

Diagram of a longitudinal section of the modified perifusion apparatus for isolated islets. Medium was drawn past the islets and through the perifusion chamber and tubing by the peristaltic action of the pump. Samples of the perfusate were collected and assayed for insulin.

silicone-treated transfer pipette. The peristaltic pump was started, and additional medium was dripped onto the filter so that the flow of fluid would permit the islets to stick to the filter. The chamber was then placed vertically in the reservoir of medium maintained at 37° C. by the water bath. The surface of the medium was gassed, and care was taken so that air bubbles did not pass through the perifusion chamber. Samples of the perifusion fluid were collected and frozen for subsequent insulin assay by the method of Wright et al.,[13] with crystalline porcine insulin used as the standard and ^{125}I-labeled porcine insulin (New England Nuclear, Boston, Massachusetts) as a tracer.

A double-chamber technic was used for most of these studies. Two identical chambers were perfused simultaneously by the same peristaltic pump, with one chamber serving to determine the effect of an experimental agent and the other chamber serving as a control. The medium was changed by transferring the perifusion chamber from one reservoir of medium to another.

Perifusion studies with calcium-free medium containing EGTA were performed in the following way: One chamber of islets was preincubated for 20 to 30 minutes in glucose-free medium containing EGTA (1 mM) with no $CaCl_2$. The islets were then exposed to the experimental agent (alloxan or D-glucose) in the absence of calcium in medium with EGTA. A parallel chamber of islets was incubated in a similar manner except for the presence of 2.5 mM $CaCl_2$ and the absence of EGTA. Insulin release during the exposure period was corrected by subtracting basal insulin release values.

Static incubation of islets. Alloxan inhibition of insulin release was determined in a static incubation system described previously.[10] Isolated islets were placed in a glass vial (11 mm. I.D. and 20 mm. high) that contained 200 μl. of medium. Each vial contained 20 to 25 islets, and 8 to 10 vials were used for each experiment. The medium was removed and added with the aid of a micropipette and a dissection microscope. The glass vial was inserted into a scintillation vial equipped with rubber stopper, gassed with O_2/CO_2 (95 per cent/5 per cent), and shaken in a Dubnoff metabolic shaker (70 to 100 cycles per minute). The islets were preincubated for 20 to 25 minutes in 200 μl. of glucose-free medium and stimulated with 200 μl. of D-glucose (27.5 mM) medium for 30 minutes at 37° C. Islets were exposed to alloxan in the following manner: The preincubation medium was removed and replaced with 200 μl. of freshly mixed medium containing alloxan. At the end of the exposure period, the alloxan medium was removed and replaced with 200 μl. of D-glucose (27.5 mM) medium for 30 minutes. Insulin release from alloxan-treated islets was compared with that from parallel groups of untreated islets. At the end of the stimulation period, the media were removed and frozen for subsequent insulin assay.

Calcium-45 uptake. Calcium-45 uptake into isolated islets was measured with a double-isotope method.[14] Twenty to twenty-five islets were preincubated (20 minutes) at 37° C. in 200 μl. of a glucose-free medium followed by incubation for five minutes in 200 μl. of medium containing an experimental agent, 2.5 mM $^{45}CaCl_2$ (6 mCi. per millimole), and 5 mM [^{3}H]sucrose (8 mCi. per millimole).

Statistics. Sigmoidal curves (see figures 5A, 6A, and 6B) were fitted to the equation $y = A/[1\text{-}Bexp(-kt)]$ or its inverse by logarithmic transformation and least squares' linear analysis.[15] Curve B in figure 5 was fitted to the equation $y = Aexp(-kt)$ by a nonlinear least squares' method.[16] Statistical analysis was performed with the unpaired Student's *t*-test and results were expressed as means ± S.E.M. with the number of observations indicated in parentheses.

Structural analysis. Using the conformations observed by x-ray diffraction studies in single crystals, the molecular structures of α- and β-D-glucose[17,18] were compared with the molecular structure of alloxan monohydrate.[19] The comparison was made with the aid of a molecular-modeling system (MMS-X), developed at Washington University, that allowed for the translation, rotation, and display of the structures of each of the compounds.

RESULTS

Effect of alloxan on insulin release. The perifusion technic with two chambers was used to determine the effect of alloxan on insulin release. In the experimental chamber, islets were preincubated for 45 minutes in glucose-free medium, exposed to alloxan (650 μM) for five minutes and then stimulated with D-glucose (27.5 mM) for 30 minutes. The control chamber of islets was treated in an identical manner except for the alloxan exposure. The concentration of alloxan was chosen from previous studies[10] in which 650 μM alloxan was the minimum concentration required to maximally (6 per cent of control) inhibit subsequent glucose-induced insulin release.

Figure 2 shows clearly that a brief burst of insulin

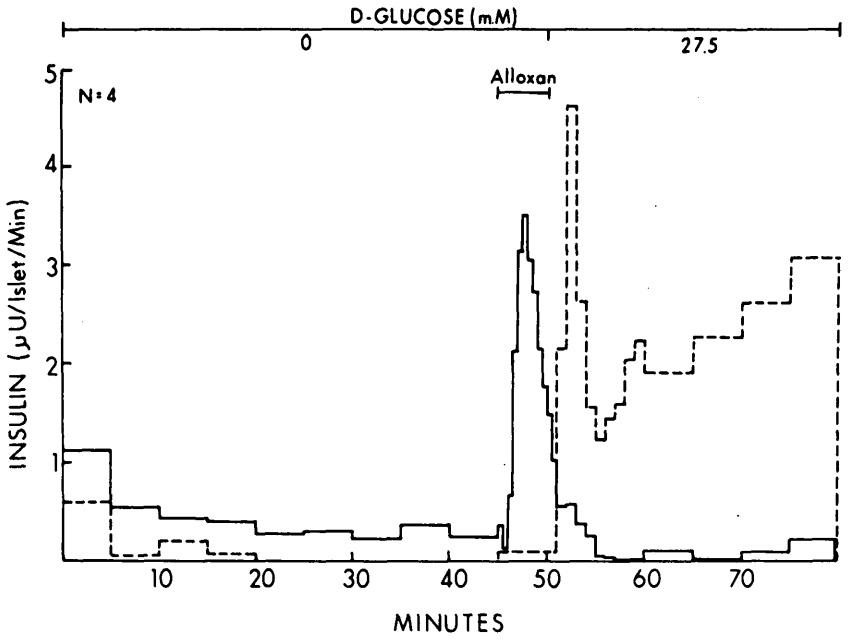

FIGURE 2

Effect of alloxan on insulin release. Islets in the experimental perifusion chamber (——) were preincubated for 45 minutes at 37° C. in glucose-free medium, exposed to freshly mixed medium with alloxan (650 μM) for five minutes, and then stimulated with D-glucose medium (27.5 mM) for 30 minutes. Islets in the control chamber (---) were incubated in an identical manner except for the alloxan exposure.

release occurred during the alloxan exposure. Insulin release during the alloxan exposure reached a maximum rate of 3.51 ± 0.50 μU. per islet per minute after 2.5 to 3.0 minutes of the exposure and then rapidly declined. The total amount of insulin released during the five-minute exposure to alloxan (0.99 ± 0.15 mU./100 islets) was significantly greater ($P < 0.005$) than the basal insulin release from the parallel chamber of untreated islets (0.05 ± 0.10 mU./100 islets).

Subsequently, both chambers of islets were stimulated with D-glucose (27.5 mM) for 30 minutes. A biphasic pattern of insulin release was observed from the group of islets that had not been exposed to alloxan. The first phase reached a maximum rate of 4.62 ± 0.88 μU. per islet per minute after two to three minutes of the glucose exposure and declined to a nadir after five to six minutes. The rate of insulin release then rose for the remainder of the D-glucose stimulation period (total release, 6.90 ± 2.30 mU./100 islets). Islets exposed previously to alloxan, however, exhibited no biphasic response to D-glucose and released insulin equivalent to basal levels (0.66 ± 0.37 mU./100 islets). Alloxan that had been allowed to decompose previously in the medium for 10 minutes at 37° C. did not stimulate insulin release from the islets (data not shown) or inhibit subsequent glucose-induced insulin release.[10] The stimulation and subsequent inhibition of insulin release appeared, therefore, to be produced by alloxan and not its decomposition product.

Repetitive alloxan pulse. We next examined whether islets exposed once to alloxan would release insulin during a second exposure to alloxan. Islets were exposed to alloxan (650 μM) for five minutes, washed in glucose-free medium for 10 minutes, and re-exposed to freshly mixed alloxan (650 μM). During the first exposure to alloxan, there was a prompt release of insulin which reached a maximum rate of 2.77 ± 0.72 μU. per islet per minute after 2.0 to 2.5 minutes of the exposure and then declined (figure 3). The total insulin released during the first five-minute exposure to alloxan was 0.67 ± 0.19 mU./100 islets; during the second exposure to alloxan, however, no insulin release was detected. These findings indicated that islets exposed to alloxan do not release insulin during a subsequent exposure to either alloxan (figure 3) or D-glucose (figure 2).

Effect of 3-0-methyl-D-glucose. Previous studies have demonstrated that the presence of 3-0-methyl-D-glucose during the alloxan exposure would protect against the subsequent inhibition of glucose-induced insulin release.[1] In the present study, 3-0-methyl-D-glucose, which has no effect on insulin release itself,[20] was used to examine the relationship of alloxan-induced insulin release to the subsequent inhibition of insulin release (figure 4). Parallel chambers of islets were exposed for five minutes either to alloxan (650 μM) alone or to alloxan (650 μM) plus 3-0-methyl-D-glucose (27.5 mM). Islets exposed to alloxan alone released a burst of insulin (total, 0.79 ± 0.16 mU./100 islets), whereas islets exposed to al-

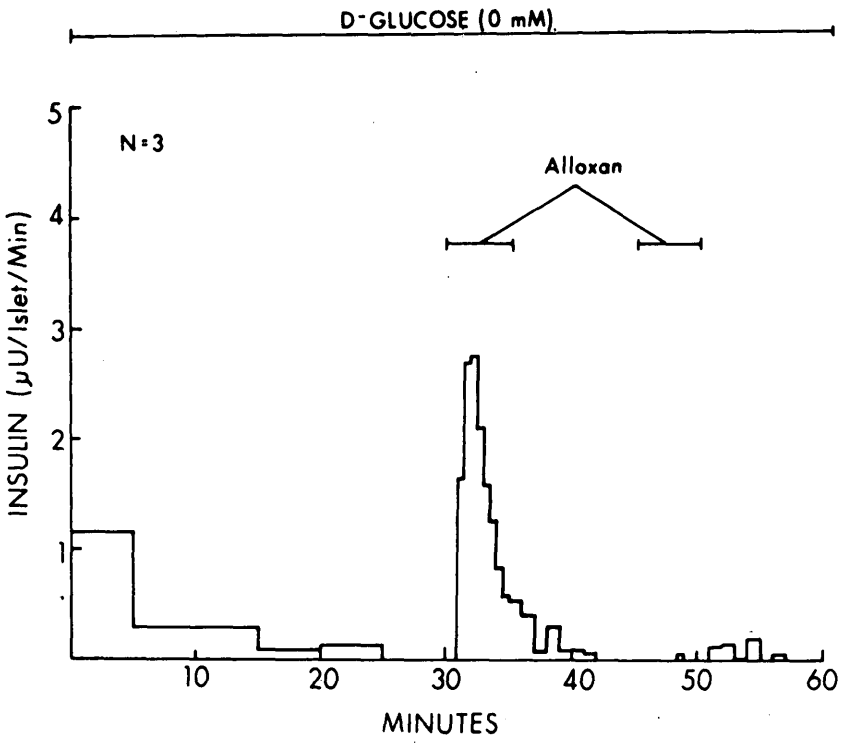

FIGURE 3

Effect of repetitive exposures to alloxan on insulin release. Islets were preincubated in glucose-free medium for 30 minutes and then exposed to freshly mixed medium with alloxan (650 μM) at 37° C. for five minutes. The islets were then incubated in glucose-free medium for 10 minutes, exposed again to alloxan (650 μM, five minutes), and finally incubated in glucose-free medium for 15 minutes.

loxan in the presence of 3-0-methyl-D-glucose continued to release insulin at basal levels (0.18 ± 0.35 mU./100 islets). These findings are consistent with the recent studies of Pagliara et al.,[5] which demonstrated that 3-0-methyl-D-glucose prevented alloxan-induced insulin release from the isolated perfused rat pancreas.

Relationship of alloxan and calcium on insulin release. Insulin release induced by D-glucose requires extracellular calcium[21,22] and is associated with an influx of

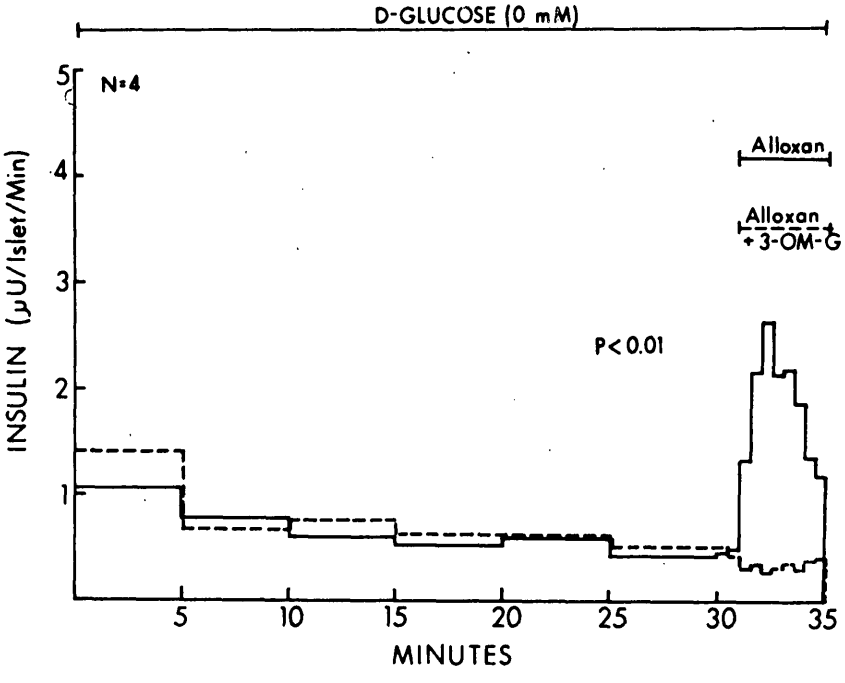

FIGURE 4

Effect of 3-0-methyl-D-glucose on alloxan-stimulated insulin release. Two groups of islets were incubated simultaneously for 30 minutes in glucose-free medium and then exposed to alloxan (650 μM) for five minutes with (---) or without (——) 3-0-methyl-D-glucose (27.5 mM) at 37° C.

^{45}Ca into the islet.[14] To determine if insulin release induced by alloxan is similarly associated with calcium, the uptake of ^{45}Ca was measured, and the calcium dependency of alloxan-induced insulin release was determined. Parallel studies in the perifusion system measured insulin release induced by alloxan (650 μM) in the presence and absence of calcium. Islets exposed to alloxan for five minutes in calcium-free medium plus EGTA (1 mM) released 71.5 per cent less insulin ($P < 0.005$ by paired t-test) than the parallel group of islets exposed to alloxan in the presence of calcium (0.10 ± 0.03 mU./100 islets vs. 0.35 ± 0.05 mU./100 islets, respectively; n = 3). In addition, other studies in the absence of calcium plus EGTA demonstrated complete inhibition of both phases of glucose-induced insulin release, which agrees with previously published results.[21,22]

The double-isotope method was used to measure the uptake of ^{45}Ca in the presence and absence of alloxan (650 μM). Table 1 shows alloxan produced a 79 per cent increase in acute ^{45}Ca uptake that was prevented by the concomitant presence of 3-0-methyl-D-glucose (27.5 mM).

Rate of alloxan inhibition of insulin release. To characterize alloxan inhibition of insulin release, its rate was determined in the static incubation system. Since D-glucose serves both as a protective agent against alloxan inhibition and as the stimulative agent for insulin release, the rate of alloxan inhibition of insulin release was determined by two methods of analysis. In the first method, islets were exposed to alloxan (650 μM) for one to five minutes and immediately incubated in D-glucose (27.5 mM) medium. Parallel groups of untreated islets were incubated in an identical manner except for the alloxan exposure. Alloxan-treated islets showed a decline in insulin release which depended on the length of exposure to alloxan (figure 5, curve A). Islets exposed to alloxan for only one minute showed insulin release near control values (92 per cent of control, $P > 0.2$); whereas, islets exposed to alloxan for five minutes released little insulin (6 per cent of control, $P < 0.001$). The apparent rate of decline in insulin release reached a maximum over the two to three minute interval, and the results were best fitted to a sigmoidal curve ($t½ = 2.5$ minutes).

TABLE 1

Effect of alloxan on acute ^{45}Ca uptake in isolated rat islets

Experimental conditions	^{45}Ca uptake (pmol/islet)
Glucose free	7.35 ± 0.86 (20)
Alloxan (650 μM)	13.16 ± 0.93 (20)*
3-0-Methylglucose (27.5 mM)	5.85 ± 0.86 (10)
Alloxan (650 μM) + 3-0-methylglucose (27.5 mM)	6.86 ± 0.88 (9)†

Calcium-45 uptake was measured for five minutes in the presence of 2.5 mM $^{45}CaCl_2$ (6 mCi. per mmol), 5 mM [^{3}H]sucrose (8 mCi. per mmol), and the compounds indicated above. Results are expressed as means ± S.E.M., and the numbers of observations are indicated in parentheses.

*$P < 0.001$.

†$P > 0.5$.

The second method of analysis was used to determine the rate of alloxan inhibition of insulin release when D-glucose was not present immediately after the alloxan exposure. In this series of experiments, islets were exposed to alloxan for one, two, or three minutes, and then washed with glucose-free medium for four, three, or two minutes, respectively, before D-glucose (27.5 mM) medium was added to the islets. In this way, the period from the onset of alloxan exposure to the addition of D-glucose medium was kept constant. Parallel groups of untreated islets were incubated in an identical manner except for the alloxan exposure and wash. Curve B in figure 5 demonstrates that islets exposed to alloxan and washed in this manner showed a rapid decline in insulin release that was dependent on the length of alloxan exposure. The most rapid decline occurred during the first minute of exposure to alloxan, when subsequent insulin release decreased to 60 per cent of control values ($P < 0.001$). The results of this second method of analysis were best fitted to a simple exponential decay curve ($t½ = 1.4$ minutes).

Marked differences were noted in the rate of alloxan inhibition of subsequent insulin release for the two methods of analysis. Islets exposed to alloxan and immediately incubated with D-glucose medium showed a slower apparent rate of decline in insulin release (figure 5, curve A) versus islets exposed to alloxan and washed before the addition of D-glucose medium (figure 5, curve B). Significantly less ($P < 0.001$) inhibition of subsequent insulin release occurred after one, two, or three minutes of alloxan exposure for islets that received D-glucose immediately after the alloxan exposure. These findings indicate that the presence of D-glucose immediately after brief alloxan exposures (one to three minutes) reversed or prevented some of the subsequent inhibition of insulin release produced by alloxan and decreased the apparent rate of inhibition.

D-glucose protection against alloxan inhibition. To examine the possible interactions of D-glucose and alloxan on insulin release, the concentration depen-

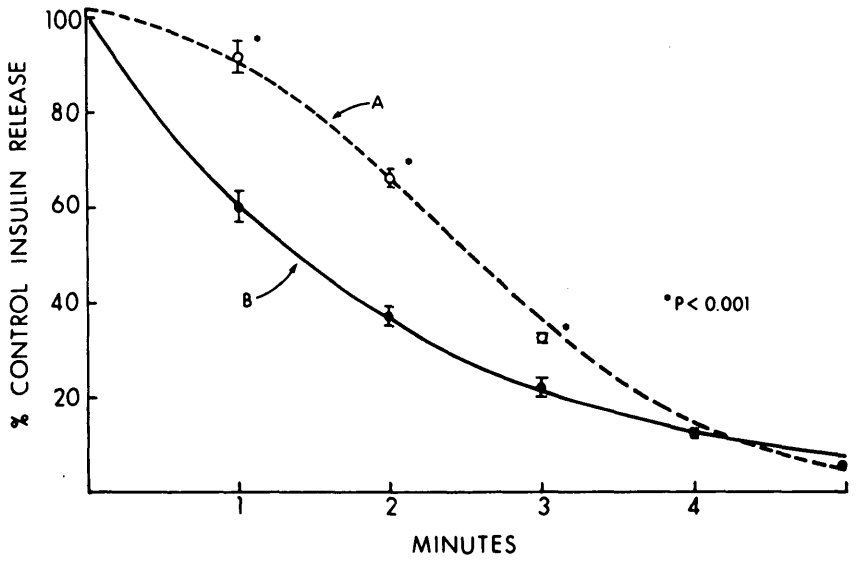

FIGURE 5

Time required for alloxan inhibition of glucose-induced insulin release. Islets were statically incubated for 20 minutes in 200 μl. of glucose-free medium at 37° C. and exposed to 200 μl. of alloxan (650 μM) in the following manner: For curve A (o---o), the islets were exposed to alloxan for the indicated intervals and then immediately incubated in 200 μl. of D-glucose medium (27.5 mM) for 30 minutes. For curve B (●—●), the islets were exposed to alloxan for one, two, or three minutes, washed for four, three, or two minutes, respectively, with 200 μl. of glucose-free medium, and finally incubated in 200 μl. of D-glucose (27.5 mM) medium for 30 minutes. The insulin released from alloxan-treated islets was compared with that from parallel untreated groups of control islets (mean rate, 3.30 μU. per islet per minute). Curves were machine drawn from least squares' analysis. Mean ± S.E.M., n = 9 to 19.

dency of D-glucose protection against alloxan inhibition of insulin release was determined. Islets were exposed for five minutes to alloxan at a concentration of 650 μM or 850 μM in the presence of a range of D-glucose concentrations (4.1 to 44 mM); the exposure medium was then removed, and the islets were incubated in D-glucose (27.5 mM) medium for 30 minutes. Previous studies[10] have demonstrated that from 650 μM to 1,250 μM alloxan produces a constant level of inhibition (6 to 8 per cent of control) of subsequent glucose-induced insulin release. Curve A in figure 6 shows that from 4.1 mM to 27.5 mM D-glucose there was an increasing level of subsequent insulin release (10 to 94 per cent of control). The glucose protection curve against alloxan (650 μM) inhibition appeared sigmoidal with an estimated half-maximal protection at 13 mM. For 850 μM alloxan, a similar curve (figure 6, curve B) was observed with an increase in the apparent half-maximal protection (26 mM) but no alteration in the final level of protection (94 per cent, at 44 mM D-glucose).

Alloxan and D-glucose structural analysis. One possible cause for the interaction of D-glucose and alloxan on insulin release is that the two compounds might

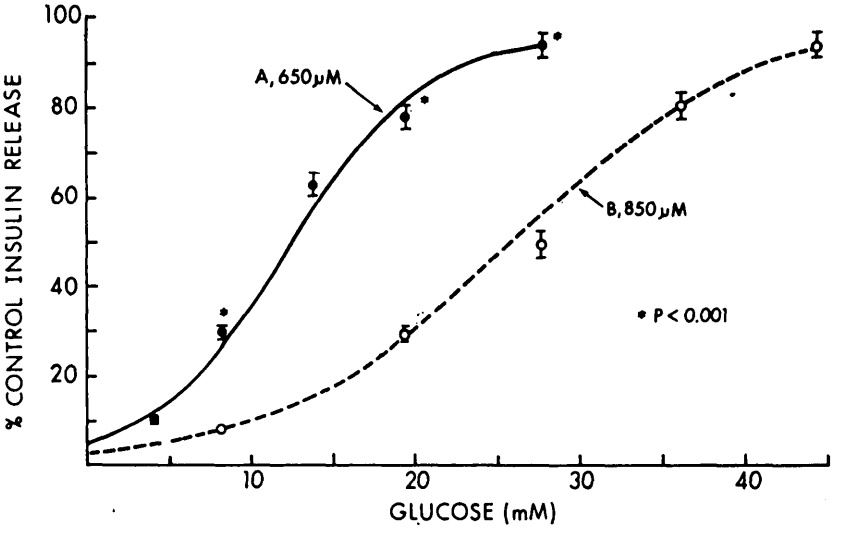

FIGURE 6

D-glucose protection against alloxan inhibition of insulin release. Islets were preincubated for 25 minutes in 200 μl. of glucose-free medium at 37° C. and then exposed for five minutes to 200 μl. medium with either 650 μM (●—●, curve A) or 850 μM (o---o, curve B) alloxan in the presence of the indicated concentrations of D-glucose (4.1 to 44 mM). At the end of the exposure period, the islets were incubated in 200 μl. of D-glucose (27.5 mM) medium for 30 minutes. The insulin released from alloxan-treated islets was compared to that from parallel untreated groups of control islets (mean rate 2.93 μU. per islet per minute). Curves were machine drawn from linear least squares' analysis. Mean ± S.E.M., n = 7 to 15.

share structural similarities. Figure 7 illustrates that alloxan (hydrated form) and D-glucose are both six-member ring compounds with a close structural similarity between oxygen atoms O_4, O_5, O_6 of alloxan and those of α-D-glucose (O_2, O_3, O_4). An analysis of the atomic structures shows that alloxan matched as closely (0.05 A variance) with α-D-glucose as β-D-glucose matched with α-D-glucose at these three oxygen positions.

DISCUSSION

Rat islets exposed to alloxan (650 μM) in a simple perifusion system released a monophasic burst of insulin that appeared similar to the first phase of insulin induced by 27.5 mM D-glucose (figure 2). The maximum rate of release occurred two to three minutes after stimulation of the islets with either alloxan or D-glucose, and the amount of insulin released during the alloxan (650 μM) exposure was about 90 per cent of the first phase of insulin release induced by 27.5 mM D-glucose. The insulin released by alloxan was, like glucose-induced insulin release, dependent upon extracellular calcium and associated with an increased uptake of ^{45}Ca. Once exposed to alloxan, however, the islets did not release insulin when stimulated again with D-glucose or alloxan (figures 2 and 3). Previous studies have shown that alloxan-treated islets are capable of releasing insulin when stimulated with tolbutamide.[1] The glucose potentiation of the tolbutamide response remained blocked, but the tolbutamide response itself appeared intact. Thus, alloxan apparently inhibited the glucose-dependent components of insulin release.

The amount of inhibition of glucose-induced insulin release was directly dependent upon the length of exposure to alloxan and was decreased by the presence of D-glucose immediately after brief (one to three minutes) alloxan exposures (figure 5). In addition, D-glucose, when present during the entire five-minute exposure to alloxan (650 μM), protected against the inhibition of insulin release in a dose-dependent manner (figure 6). With an increase in the alloxan concentration from 650 μM to 850 μM, there was an increase in the concentration of D-glucose at half-maximal protection (13 mM to 26 mM) but no alteration in the final level of protection (94 per cent). These findings suggest the protection by D-glucose against alloxan inhibition was similar to competitive enzyme inhibitors.

Additional information is known about the interactions of D-glucose and alloxan on the β-cell. D-glucose does not protect against alloxan inhibition by decreasing the total uptake of alloxan; indeed, recent studies have shown that D-glucose increases the uptake of [2-^{14}C]alloxan by isolated islets.[23] Nor does alloxan inhibit insulin release simply by inhibiting the uptake of D-glucose; the rate of D-glucose transport in the islet is unaltered after the alloxan exposure.[24] The ability of 3-0-methyl-D-glucose, a nonmetabolized hexose, to prevent alloxan's actions on insulin release (stimulation and subsequent inhibition) and the increased ^{45}Ca uptake (table 1) argues against metabolism being a direct influence on hexose protection. Both D-glucose and 3-0-methyl-D-glucose do, however, share a common carrier in the islet.[3] Similarly, caffeine, theophylline, and cytochalasin B inhibit the carrier for D-glucose in the islet and protect against alloxan inhibition if insulin release.[4,25,27] The site of protection by these agents (3-0-methyl-D-glucose, caffeine, theophylline, cytochalasin B) may be near or structurally related to the hexose transport carrier on the islet cell membrane.

D-glucose and alloxan may interact with the insulin release mechanism because they are structurally similar (figure 7). Alloxan at positions O_4, O_5, and O_6 and α- or β-D-glucose at positions O_2, O_3, and O_4 show little variance in the orientation of their oxygen atoms. Recently, ninhydrin, which in the anhydrous form is almost identical to alloxan in the orientation of its oxygen atoms,[28] has been shown to inhibit subsequent glucose-induced insulin release.[29] Alloxan and ninhydrin may share their effects on insulin release because they share with D-glucose a similar orientation of their oxygen atoms.

Current evidence indicates, then, that alloxan reacts with the insulin release mechanism in two stages—first, stimulation; second, inhibition of insulin release. A similar two-stage effect has been observed on catecholamine release by the 2-haloalkylamines, which stimulate and then inhibit the alpha-adrenergic receptor.[30] Nickerson[31] has argued that the two stages of action on catecholamine release represent an early competitive association of the 2-haloalkylamines with the receptor and a subsequent noncompetitive inhibition that results from a chemical reaction with the receptor. In an analogous manner, alloxan could initiate insulin release by associating with a receptor during its first stage of action and chemically react with this receptor during the second stage to prevent further stimulation with alloxan or D-glucose. The protection by D-glucose during the

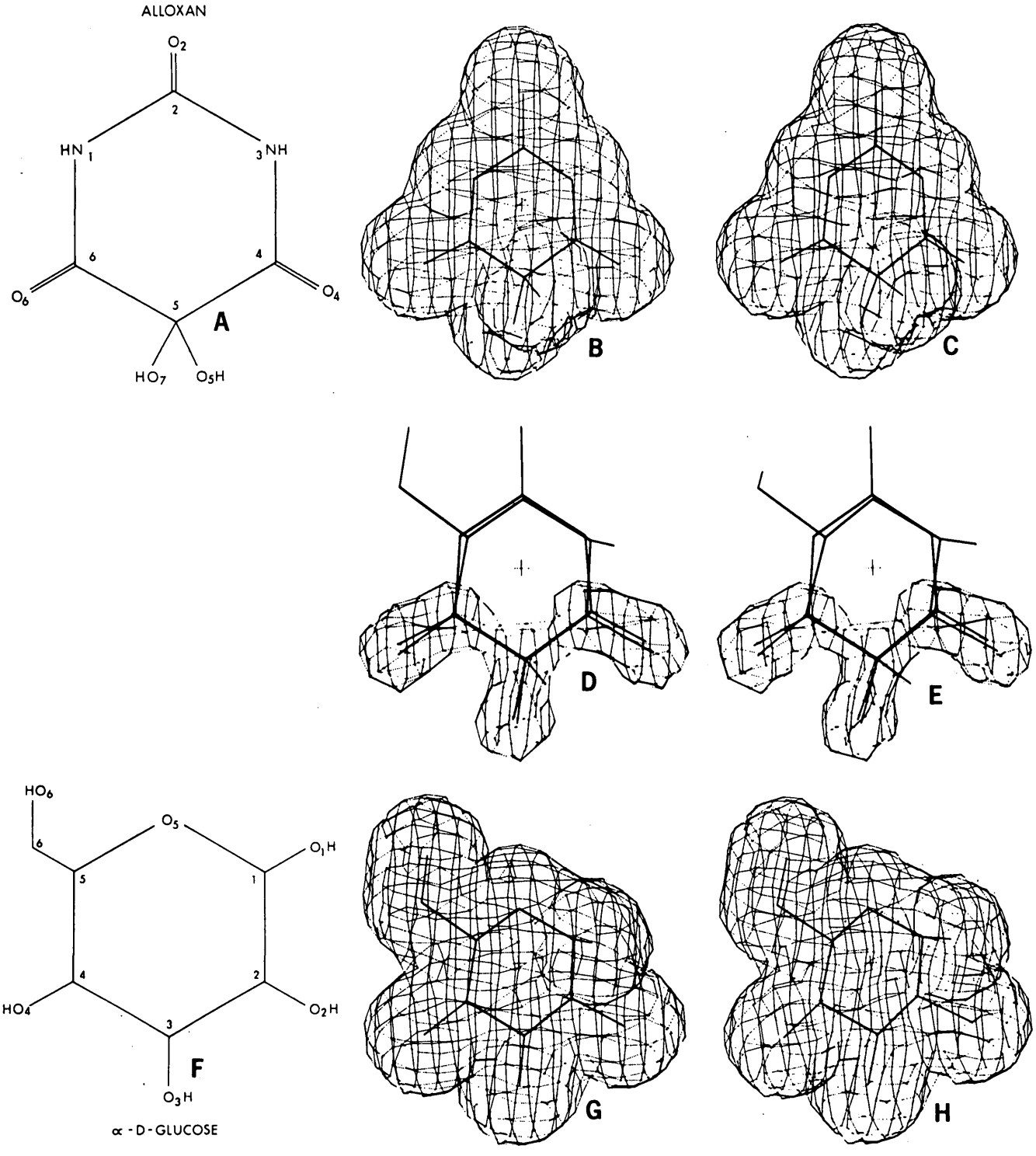

FIG. 7. Stereoscopic comparison of the molecular structures of alloxan (hydrated form) and α-D-glucose. The upper three figures are the chemical structure for alloxan monohydrate (A), and left (B) and right (C) are stereoscopic models of alloxan drawn by a computer. The darker internal lines represent the interatomic axes, and the light outer lines represent the approximate van der Waals radii. The lower three figures are the chemical structure for α-D-glucose with some of the hydrogens deleted for clarity (F), and left (G) and right (H) are computer-drawn stereoscopic models. In the center (D and E) is a stereoscopic presentation of alloxan and α-D-glucose overlapped, with the region of particularly close approximation outlined. Instructions for stereoscopic viewing, which include a stereoscopic viewer, have been published.[32]

first stage of alloxan's action on insulin release may result from the displacement of alloxan from a common receptor, which initiates insulin release. Studies are in progress with [2-^{14}C]alloxan and [^{3}H]ninhydrin to examine directly the existence of receptors for D-glucose.

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