Pancreatic Beta Cell Toxicity by Streptozotocin Anomers

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SUMMARY

D-glucose in the pyranose (ring) form exists as two anomers. The α-anomer is more effective than the β-anomer in promoting insulin secretion, suppressing that of glucagon, and protecting β-cells against alloxan toxicity. Streptozotocin (SZ), a beta cell toxin, is composed of a cytotoxic moiety, 1-methyl 1-nitrosourea, attached to carbon-2 of glucose and exists as either of two anomers in the pyranose form. In 24-hour-fasted male rats, predominantly α- or predominantly β-SZ was injected intravenously and plasma glucose levels were obtained 48 hours later. The α-anomer produced significantly greater β-cell necrosis at doses of 30, 35, and 40 mg./kg. body weight. At higher doses, no differences between the α and β anomers were observed. 3-O-Methyl glucose (3-OMG) protected against both SZ anomers; however, the α-SZ remained more toxic. Larger doses of glucose protected against the lower doses of SZ and, under such conditions, the individual glucose anomers appeared equally potent. Finally, mannitol at comparable molar concentrations was ineffective in protecting against the SZ toxicity.

This study suggests that streptozotocin's beta cell toxicity is mediated through recognition by the beta cell. In addition, 3-OMG and, to a lesser but significant extent, glucose were shown to protect against the streptozotocin toxicity, whereas mannitol did not. DIABETES *26*:**1120-24, December, 1977.**

Streptozotocin (SZ) is derived from Streptomyces acromogenes and has antibacterial, oncogenic, and cytotoxic properties, particularly to the pancreatic beta cell.[1-4] The compound consists of a 1-methyl 1-nitrosurea moiety linked to carbon-2 of D-glucose. It probably exists in the pyranose form, with two anomers at carbon-1 similar to those of D-glucose. The manner in which SZ produces beta cell necrosis is unknown but is thought to be mediated from within the beta cell itself.[5] Furthermore, it has been suggested that the glucose component of SZ enhances the uptake of SZ into islet cells, where the cytotoxicity of the methylnitrosurea can exert its full effect.[6,7]

Recent studies have demonstrated that the α-anomer of glucose is more effective than the β-anomer in stimulating insulin release, in suppressing glucagon release from the alpha cell, and in protecting against alloxan-induced beta cell necrosis.[8-13] Accordingly, it has been proposed that α-glucose has a greater affinity for a "receptor site" on or within the beta cell.

In isolated pancreatic islets, isotopically labeled β-D-glucose is incorporated at twice the rate of α-D-glucose.[14] Other observations, using countertransport diffusion in pancreatic islets, suggest that the two anomers are equally active in penetrating the beta cell.[15] Thus, the two anomers appear to enter beta cells at the same rate, while according to one report β-D-glucose is accumulated preferentially in metabolic intermediates. Thus, α-glucose may act as a preferential stimulator of insulin release by a "receptor" interaction and β-D-glucose may be metabolized more rapidly, although a recent report suggests that α-D-glucose may be metabolized more rapidly.[16]

Since the glucose moiety of the SZ molecule is thought to be essential for pancreatic beta cell toxicity, α- and β-SZ anomers were administered to determine if they were equally effective in producing pancreatic beta cell necrosis in the rat. Furthermore, 3-O-methyl D-glucose (3-OMG), a nonmetabolized analogue of glucose, was administered prior to injection of the SZ anomers in order to study the relation-

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Accepted for publication July 12, 1977.

ship between the anomers of SZ and the protection against beta cell toxicity afforded by 3-OMG. Also studied were the protective properties of high doses of mutarotated D-glucose, as well as the individual α- and β-D-glucose anomers.

The results of these studies support the concept that the initial mediation of streptozotocin toxicity is at a receptor site, possibly on the surface of the beta cell. Also, a caveat for future research projects can be sounded: henceforth, the relative proportions of the SZ anomers in a given preparation should be characterized and recorded in all studies of SZ-induced beta cell necrosis.

MATERIALS AND METHODS

The experimental techniques for the intravenous cannulation of rats and sampling of blood have been described.[8] Male rats (Charles River strain of Sprague-Dawley), weighing 180-200 gm., were fasted for 24 hours. α-Streptozotocin (lot 11676-RLH-112 U-9889, assayed as 90 per cent α and 10 per cent β) and β-streptozotocin (lot 9681-GGS-118FI, U-9889, assayed at 25 per cent α and 75 per cent β) were obtained from the Upjohn Company, Kalamazoo, Mich. The crystalline streptozotocin (SZ) anomers were dissolved in citrate buffer (pH adjusted to 4.7) 30-45 seconds prior to injection. 3-O-methyl D-glucose (3-OMG) (Sigma Chemical, St. Louis, Mo.) was dissolved in saline 24 hours prior to administration to ensure complete mutarotation. Doses of 3-OMG of 0.55, 0.83, or 1.1 mM/200 gm. body weight were injected in volumes of 1 ml. α-glucose crystals (lot 0916-1190, listed as 97.6 per cent α and 2.4 per cent β) and β-glucose crystals (lot 0526-0810, listed as 99.2 per cent β and 0.8 per cent α) were obtained from Sigma. Mutarotated D-glucose (66 per cent β and 34 per cent α) was administered from a stock solution of 50 per cent W/V obtained from Abbott Laboratories (44-314-DK). D-glucose and the glucose anomers (2.7 mM in volumes 1 ml./200 gm. body weight) were injected, as were other substances, such as mannitol (1.5 ml.) and 3-OMG, over a 30-second period and followed within 30 seconds by the administration of the SZ anomers. The glucose anomers were dissolved immediately prior to use. Approximately three minutes for the α- and 45 seconds for the β-glucose elapsed before complete dissolution occurred. Mannitol (lot 1236-1650) was obtained from Sigma Chemical and was administered in some experiments prior to the SZ anomers, at a dose of 2.7 mM in 1.5 ml./200 gm. body weight. Following the injection of test substances, an additional 0.3 ml. of isotonic saline was injected to flush the tubing. The animals were then allowed free access to food and water. Forty-eight hours later, tail-blood samples were centrifuged, and plasma glucose (PG) measured in 10-μl. samples with a Beckman Glucose Analyzer. The animals were killed by decapitation, and pancreases were fixed in Bouin's solution prior to paraffin embedding and aldehyde fuchsin staining of histologic sections. Statistical analyses were performed by means of an unpaired *t*-test.[17]

RESULTS

Figure 1 records PG levels 48 hours following the intravenous administration of predominantly α- or predominantly β-SZ. Increasing concentrations of SZ produced progressively greater beta cell necrosis, as manifested by increasing glucose concentrations. Significant differences were observed between the two SZ anomers at doses of 30, 35, 40, and 45 mg./kg., but not at lower or higher doses. Light-microscopic studies of the pancreatic islets demonstrated more severe beta cell necrosis in rats receiving the α-anomer of

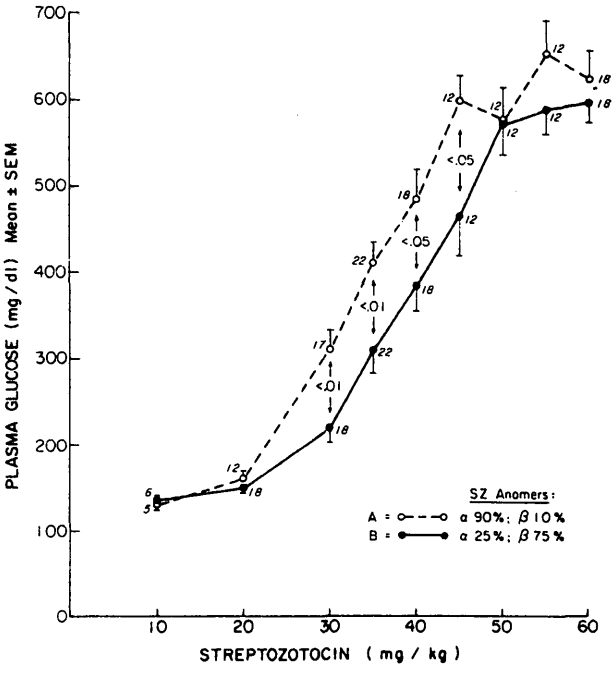

FIG. 1. Plasma glucose levels (Mean ± S.E.M.) 48 hours following the intravenous injection of α- and β-SZ at 10-60 mg./kg. body weight in 24-hour-fasted rats. The italicized numbers represent the number of animals in each group. Statistical analyses (p values) performed by means of unpaired *t*-test.

SZ at doses of 30 and 40 mg./kg. Although histologic differences were occasionally equivocal, the β-anomer never produced greater beta cell toxicity than the α-anomer.

Figure 2 illustrates the relative protection afforded by 3-OMG at 0.55, 0.83, and 1.1 mM/200 gm. body weight against both α and β SZ (60 mg./kg. body weight). At this dose of SZ, the effects of α- and β-SZ anomers were not significantly different, and hyperglycemia was maximal (figure 1). However, when the dose of 3-OMG was progressively increased, a relative protection was observed, and a greater toxicity of the α-SZ became apparent.

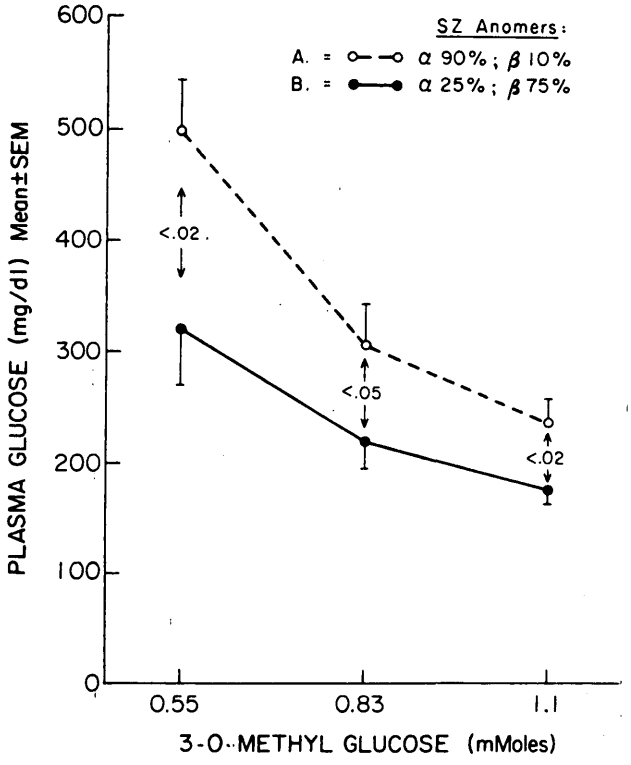

FIG. 2. Plasma glucose levels (Mean ± S.E.M.) 48 hours following the administration of 3-OMG at 0.55, 0.83, and 1.1 mM per rat body weight just prior to the intravenous injection of streptozotocin anomers (60 mg./kg. body weight) in 24-hour-fasted rats. Statistical analyses (p values) performed by means of an unpaired *t*-test. (n = 10).

Figure 3 illustrates the PG levels of rats 48 hours following the administration of mutarotated D-glucose at 2.7 mM/200 gm. body weight, followed by increasing doses of the SZ anomers. For comparison, we have included the PG levels of animals receiving the SZ anomers alone (figure 1). The prior injection of mutarotated glucose provided significant protection against SZ at doses of 30, 40, and 50 mg./kg.

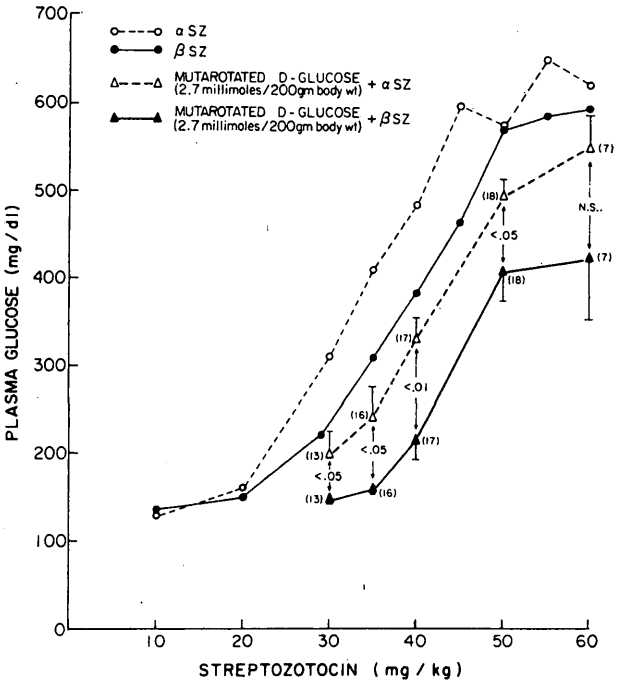

FIG. 3. Plasma glucose levels (Mean ± S.E.M.) 48 hours following the intravenous injection of SZ anomers in 24-hour-fasted rats (from figure 1). Also illustrated is the PG response of animals pretreated with mutarotated D-glucose (2.7 mM per rat) prior to injection of the SZ anomers. Italicized numbers represent the number of animals in each group. Statistical analyses (p values) performed by means of unpaired *t*-test.

Table 1 shows the results obtained when the individual α- and β-D-glucose anomers (2.7 mM/200 gm. body weight) were administered prior to the α- or β-SZ anomers at 40 mg./kg. The more diabetogenic effect of α-SZ is again noted. Although the glucose anomers appeared to produce protection, only the α-glucose anomer provided a significant difference when compared with the mutarotated glucose or the β-D-glucose. Furthermore, the α-glucose protected only against the β-SZ.

To exclude the possibility that protection was due to an osmotic effect, mannitol (2.7 mM/200 gm. body weight) was administered prior to α- and β-SZ (30-40 mg./kg.). Table 2 shows the results obtained when the SZ anomers were administered alone or following the injection of mannitol. Lower glucose values were frequently observed after mannitol, but the differences were not significant, and much less than those observed after the injection of glucose or 3-OMG.

DISCUSSION

It has been proposed previously that the glucose

TABLE 1

Predominant D-glucose anomers (2.7 mM/200 gm. body weight)	Streptozotocin anomers (40 mg./kg. body weight)	Plasma Glucose Mean	± S.E.M.	N	P*
α glucose	α SZ	288	35	20	N.S.
β glucose	α SZ	288	33	19	N.S.
Mutarotated glucose	α SZ	331	24	17	
α glucose	β SZ	168	9	22	> 0.05
β glucose	β SZ	174	13	27	
Mutarotated glucose	β SZ	215	21	17	N.S.

*P = Unpaired Student *t*-test.

moiety of streptozotocin (SZ) is important in producing its diabetogenic activity[5] by acting as a vehicle for contact with, or transport across, the beta cell plasma membrane. The diabetogenic action of SZ is then thought to be produced by the N-nitrosomethylurea acting within the beta cell and causing, among other effects, a reduction in the levels of pyridine nucleotide. However, recent observations have shown that damage to the β-cells of Chinese hamsters, in vitro[7] and in vivo,[6] occurs after the administration of N-nitrosomethylurea, the aglucone derivative of streptozotocin. Thus, although the glucose residue of streptozotocin is not absolutely necessary for the induction of β-cell necrosis, the glucose moiety does appear to increase the sensitivity and specificity of the cytotoxic action.

The injection of nicotinamide, simultaneously with or up to two hours after the administration of SZ, provides almost complete protection of the pancreatic β-cells.[18-20] However, 2-deoxyglucose and 3-OMG appear to provide protection only when injected prior to or simultaneously with SZ.[18-21] These results suggest that nicotinamide provides protection at a step later in the chain of events, while 2-deoxyglucose and 3-OMG exert their actions at an earlier stage (e.g., at the "receptor" or cell surface).

TABLE 2

Streptozotocin anomers	Mannitol (2.7 mM/200 gm. body weight)	Plasma glucose Mean	±S.E.M.	N	P*
α SZ 30 mg./kg.	−	310	±23	17	
α SZ 30 mg./kg.	+	278	±29	19	N.S.
β SZ 30 mg./kg.	−	219	±16	18	
β SZ 30 mg./kg.	+	188	±17	19	N.S.
α SZ 40 mg./kg.	−	484	±30	18	
α SZ 40 mg./kg.	+	468	±27	9	N.S.
β SZ 40 mg./kg.	−	386	±29	18	
β SZ 40 mg./kg.	+	312	±39	10	N.S.

*P = unpaired Student *t*-test.

In this study, α-SZ caused more severe hyperglycemia than β-SZ at doses between 30 and 45 mg./kg. body weight. At lower or higher doses there were no differences between the SZ anomers. The protection afforded by 3-OMG appears to dissociate the previously undifferentiated toxicity of the SZ anomers at 60 mg./kg., with α-SZ being more toxic. These data suggest that the site of protection appears to have a higher affinity for the α-glucose moiety of SZ and that 3-OMG acts protectively at this site.

Previous studies have failed to show glucose protection against SZ in the pancreatic beta cells, although glucose protection against SZ toxicity has been found in human red blood cells.[22] In our experiments, the protection afforded by high concentrations (2.7 mM) of D-glucose against smaller doses of SZ suggests that glucose has a small degree of protective capacity. The glucose anomers were studied to characterize further the specificity of this protection. Only α-glucose produced a significant difference when compared with mutarotated D-glucose or β-glucose. Furthermore, α-glucose was protective only against β-SZ. The large standard errors precluded significance of the other comparisons. Nevertheless, the greater effectiveness of β-glucose compared with mutarotated glucose (table 1) is surprising and unexplained. In order to assure that the glucose concentration was not producing an osmotic protective effect, mannitol was administered at an equimolar dose and no significant protection was demonstrated.

Since α-SZ had greater beta cell toxicity than β-SZ, the percentage of α-SZ in lots prepared in 1970-1975 by the Upjohn Company was investigated. The proportion of α-SZ in these varied from 25 to 90 per cent. Thus, one lot of SZ may be more potent than another and could provide a source of undesirable variation in studies of beta cell toxicity. In dissolving the crystalline SZ, the compound is placed into citrate buffer, pH 4.0, at room temperature. The rate of mutarotation was such that equilibration occurred at approxi-

mately one hour. Although it is recommended that SZ be used rapidly (within 10 minutes) after dissolution, variations in the elapsed time before injection could result in possible variations in apparent potency. Thus, in future studies, standardization of the SZ in relation to the anomerization of the glucose moiety will be necessary in order to achieve reproducibility of SZ action from lot to lot.

ACKNOWLEDGMENTS

We wish to acknowledge the excellent technical assistance of Ms. Anne Muldoon and Mr. David W. Glynn and the preparation of the manuscript by Ms. Priscilla Gobron.

This work was supported by USPHS grants AM-15191 and AM-15155, and grants from the American Diabetes Association and the Juvenile Diabetes Foundation.

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