Effect of Streptozotocin on Glucose-induced Insulin Secretion by Isolated Islets of Langerhans

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SUMMARY

The present experiments were designed to investigate the immediate action of streptozotocin upon the beta cell by measuring its effect upon glucose-induced release by isolated islets of Langerhans. The results demonstrated a dose-related inhibition of glucose-induced secretion during the first two hours of incubation, with the inhibition most marked in the second hour. The same level of inhibition was achieved in the second hour regardless of whether the drug was present for both hours or for just the first hour.

Nicotinamide showed a dose-related protection against the inhibitory action of streptozotocin whereas nicotinic acid did not. Further, nicotinamide showed a protective trend even when added one hour after streptozotocin—indicating that no irreversible change in the insulin secretory mechanism had been elicited over the first hour of incubation. An enhancement of glucose-induced secretion was observed with nicotinamide alone. Nicotinamide was not able to protect against the inhibitory action of mannoheptulose and the latter had no significant effect upon streptozotocin's action.

It is concluded that the early hyperglycemia induced by streptozotocin is the result of a direct action of streptozotocin on the beta cell and that the same biochemical lesion may be responsible for both the impairment of insulin secretion and eventual beta-cell destruction. Finally, the demonstration of a protective action of nicotinamide at the site of the beta cell suggests that the nicotinamide adenine dinucleotide (NAD) level of the beta cell may be critical for maintenance of normal function. DIABETES *20*:513-18, August, 1971.

Streptozotocin, an antitumor and diabetogenic agent composed of glucose and 1-methyl-1-nitrosourea (MNU),[1] has been shown to produce irreversible hyperglycemia in rats, dogs, mice and rhesus monkeys.[2-4] Guinea pigs pretreated with insulin also develop streptozotocin-induced diabetes.[5] Diabetes onset following intravenous streptozotocin follows a triphasic pattern with an early hyperglycemia (peaking at the second hour) followed by hypoglycemia (most marked at the seventh hour) and, finally, by permanent hyperglycemia by the twenty-fourth hour.[6,7]

The work of Junod et al. clearly assigns the permanent diabetogenic property of streptozotocin to its β-cell cytotoxic action, whereas the mechanism behind the early hyperglycemic phase is yet to be elucidated.

Schein et al. observed that nicotinamide protects against the diabetogenic effect of streptozotocin.[8-10] Further studies have demonstrated that nicotinamide could be administered as late as two hours after streptozotocin without loss of its protective effect.[11-13]

The intimate mode of action of both streptozotocin and nicotinamide has yet to be elucidated. Schein et al. reported that both streptozotocin and MNU lower liver nicotinamide adenine dinucleotide (NAD) levels, and that nicotinamide antagonizes these effects.[8,9] Whether comparable changes occur in the β cell and might account for the corresponding alteration of islet function, remains to be investigated. In the present study, the direct and immediate influence of streptozotocin upon the β cell's function was investigated by examining its effect upon glucose-induced insulin release in isolated islets of Langerhans.

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TABLE 1

Effect of varying concentrations of streptozotocin (St) on insulin secretion.
Mean ± S.E.M. expressed as per cent of mean control rate found within the same experiment with statistical significance of each compared with its own mean control.

First hour				Second hour			
Drug and dose (mg./ml.)	Insulin output Mean ± S.E.M.*	No. flasks	p†	Drug and dose (mg./ml.)	Insulin output Mean ± S.E.M.*	No. flasks	p†
St (0.05)	93.0 ± 12.8	(7)	> 0.7	nil	76.3 ± 12.3	(7)	> 0.3
St (0.2)	86.0 ± 10.6	(6)	> 0.4	nil	59.3 ± 9.4	(6)	< 0.05
St (0.5)	66.4 ± 2.4	(39)	< 0.001	nil	21.8 ± 2.4	(32)	< 0.001
St (1.0)	51.7 ± 5.8	(20)	< 0.001	nil	14.7 ± 5.6	(6)	< 0.001

*Mean ± S.E.M. expressed as per cent of mean control rate of secretion.
†P-values obtained by comparison with mean control secretion in each experiment.

METHODS

Islets were isolated from fed, male albino rats (300-350 gm.) by the method of Lacy and Kostianovsky.[14] Groups of eight islets were transferred to micro-incubation flasks and incubated in a bicarbonate-buffered medium (1.0 ml.) containing albumin (5 mg./ml.) and equilibrated with oxygen (95 per cent) and carbon dioxide (5 per cent), according to a method described previously.[15] Control media contained glucose alone (3 mg./ml.). Test media contained glucose (3 mg./ml.) together with one or the combination of the following agents: streptozotocin (Upjohn lot #9164-VDV-136),* nicotinamide (Sigma), nicotinic acid (Sigma), and mannoheptulose (Nutritional Biochemicals Corporation). The pH of test media was adjusted to be equal to that of the control media (pH 7.4). The flasks were then incubated for two successive one-hour incubations at 36° C. in a Dubnoff metabolic shaker. After the first hour the media were removed and either control or test media added for the second-hour incubation. The insulin content of the media was assayed according to the immunoassay of Wright and Malaisse[16] as applied to isolated islets.[15]

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Presentation of results

In the control media containing glucose alone (3 mg./ml.), the mean absolute rate of insulin release averaged 187 ± 7 and 186 ± 6 μU./islet per hour ($n_1 = 102$; $n_2 = 83$), respectively, for the first and second hour of incubation. In each experiment, five to seven groups of eight islets each were incubated in such control media; an equal number of measurements were performed in test media containing both glucose and some other agent or combination of agents. Thus, in all cases, the difference between control and experimental values was tested by comparison of groups with equal size. In the tables, the rates of insulin secretion found in the test media are expressed in per cent of the mean control value (glucose alone) found within the same experiment and during the same period of incubation.

RESULTS

Table 1 contains data for various concentrations of streptozotocin in experiments in which the drug was present only during the first hour of incubation. Increasing concentration of streptozotocin from 0.05 mg./ml. to 1.0 mg./ml. yielded a dose-related inhibition of glucose-induced insulin secretion. The minimum amount of streptozotocin necessary to elicit significant inhibition was 0.2 mg./ml. which depressed secretion to 59.3 per

TABLE 2

Effect of streptozotocin (St) when present during both hours of incubation

First hour				Second hour			
Drug and dose (mg./ml.)	Insulin output Mean ± S.E.M.*	No. flasks	p†	Drug and dose (mg./ml.)	Insulin output Mean ± S.E.M.*	No. flasks	p†
St (0.2)	91.9 ± 11.6	(14)	> 0.6	St (0.2)	50.8 ± 8.1	(6)	< 0.02
St (0.5)	61.7 ± 4.7	(28)	< 0.001	St (0.5)	23.1 ± 2.9	(14)	< 0.001

*Mean ± S.E.M. expressed as per cent of mean control rate of secretion.
†P-values obtained by comparison with mean control secretion in each experiment.

TABLE 3

Effect of nicotinamide (N) and nicotinic acid (N.A.) on the inhibitory action of streptozotocin (St)

First hour				Second hour			
Drug and dose (mg./ml.)	Insulin output Mean ± S.E.M.	No. flasks	p	Drug and dose (mg./ml.)	Insulin output Mean ± S.E.M.	No. flasks	p
St(0.5) + N(0.5)	96.4 ± 7.6	(5)	> 0.6	nil	*45.0 ± 9.5	(5)	< 0.01
St(0.5) + N(1.25)	125.0 ± 6.6	(5)	< 0.02	nil	*67.0 ± 5.8	(5)	< 0.01
St(0.5) + N(2.5)	131.0 ± 2.5	(7)	< 0.05	nil	*79.2 ± 2.6	(7)	> 0.05
St(0.5) + N(2.5)	128.8 ± 5.5	(13)	< 0.001	St(0.5) + N(2.5)	†110.2 ± 5.8	(14)	> 0.1
St(0.5) + N.A.(2.5)	72.2 ± 2.6	(7)	< 0.02	nil	‡30.4 ± 4.9	(7)	< 0.001

*Compared with 21.8 per cent from table 1, $p < 0.01$, p. < 0.001, p. < 0.001.
†Compared with 23.1 per cent from table 2, $p < 0.001$.
‡Compared with 21.8 per cent from table 1, $p > 0.1$.

cent of control in the second hour. A dose of 0.5 mg./ml. was required to demonstrate significant inhibition in the first hour of incubation. The degree of inhibition was consistently more marked during the second hour; thus, the rate of insulin release ranged, for the concentrations used, from 14.7 per cent to 76.3 per cent of control during the second hour, as compared with 51.7 per cent to 93.0 per cent in the first hour. Table 2 contains similar data for two concentrations of streptozotocin in experiments in which the drug was present during both hours of incubation. By comparing the appropriate data from tables 1 and 2, one finds the same level of inhibition regardless of whether the drug was present for both hours or just for the first hour ($p > 0.5$, $p > 0.7$).

Table 3 summarizes data for the combination of nicotinamide or nicotinic acid with streptozotocin. Three nicotinamide concentrations, 0.5 mg./ml., 1.25 mg./ml., and 2.5 mg./ml. combined with a fixed concentration of streptozotocin (0.5 mg./ml.) demonstrated dose-related protection from the inhibition of glucose-induced secretion in the second hour. The second hour secretion rates for these combinations were 45.0 per cent, 67.0 per cent and 79.2 per cent respectively. All these rates of secretion are significantly higher than 21.8 per cent (table 1), the level achieved in the second hour using streptozotocin alone ($p < 0.01$, $p < 0.001$, $p < 0.001$). Similarly, the combination of nicotinamide (2.5 mg./ml.) and streptozotocin (0.5 mg./ml.) during both hours of incubation gave a second-hour rate of secretion of 110.2 per cent as compared to 23.1 per cent (table 2) for the comparable dose of streptozotocin alone ($p < 0.001$). Nicotinic acid (2.5 mg./ml.), however, did not protect against streptozotocin's inhibitory action on glucose-induced secretion. The second-hour level of secretion for the combination was 30.4 per cent of its control as compared with 21.8 per cent (table 1) for streptozotocin alone ($p > 0.1$).

Table 4 contains data for control experiments using nicotinamide (2.5 mg./ml.) alone during either the first or both hours of incubation. Whenever present in the incubation medium, nicotinamide significantly enhanced glucose-induced secretion. After exposure to nicotinamide, the islets secreted insulin at a normal rate when transferred to a control medium for the second hour of incubation. During the first hour of incubation, the rates of secretion induced by glucose in the presence of nicotinamide alone (table 4) were not significantly different from those observed in the simultaneous presence of nicotinamide and streptozotocin (table 3). Even during the second hour of incubation, the rates of secretion after exposure to the combination of strepto-

TABLE 4

Effect of nicotinamide (N) on glucose-induced secretion

First hour				Second hour			
Drug and dose (mg./ml.)	Insulin output Mean ± S.E.M.	No. flasks	p	Drug and dose (mg./ml.)	Insulin output Mean ± S.E.M.	No. flasks	p
N (2.5)	*149.8 ± 9.6	(6)	<0.01	nil	†90.6 ± 7.8	(6)	> 0.4
N (2.5)	*132.8 ± 7.8	(13)	< 0.02	N (2.5)	†130.2 ± 7.5	(13)	< 0.02

*Compared with 131.0 per cent and 128.8 per cent from table 3, respectively, $p > 0.1$, $p > 0.6$.
†Compared with 79.2 per cent and 110.2 per cent from table 3, respectively, $p > 0.3$, $p < 0.05$.

TABLE 5

Effect of nicotinamide (N) added one hour after streptozotocin (St)

First hour				Second hour			
Drug and dose (mg./ml.)	Insulin output Mean ± S.E.M.	No. flasks	p	Drug and dose (mg./ml.)	Insulin output Mean ± S.E.M.	No. flasks	p
St (0.5)	69.8 ± 3.2	(7)	< 0.01	N (2.5)	61.1 ± 6.1	(7)	< 0.001

zotocin and nicotinamide, 79.2 per cent and 110.2 per cent (table 3) were either not significantly different or only slightly less than the rates of the corresponding nicotinamide controls, 90.6 per cent and 130.2 per cent respectively ($p > 0.3$, $p < 0.05$). All these data indicate that in the presence of nicotinamide, the inhibitory effect of streptozotocin on glucose-induced secretion was either markedly reduced or completely abolished.

Table 5 contains the results of an experiment in which streptozotocin (0.5 mg./ml.) was present alone in the first hour and followed by nicotinamide (2.5 mg./ml.) alone in the second hour. The second hour secretion was 61.1 per cent as compared to 21.8 per cent (table 1) for the same dose of streptozotocin alone, and thus indicated a reversing trend ($p < 0.001$) as a result of adding nicotinamide.

Table 6 summarizes the data for two experiments in which mannoheptulose, a well-known inhibitor of glucose-induced secretion,[17] was combined with either nicotinamide or streptozotocin. First, to determine whether nicotinamide's protective action was specific for streptozotocin, its effect on the inhibitory action of mannoheptulose was examined over one ninety-minute incubation. Nicotinamide (2.5 mg./ml.) failed to overcome the inhibitory effect of mannoheptulose (2.1 mg./ml.). Second, since mannoheptulose is reported to block the phosphorylation of glucose,[18] the possibility that streptozotocin needs to be phosphorylated to exert its effect was examined. Mannoheptulose (2.1 mg./ml.) was combined with streptozotocin (0.5 mg./ml.) in the first hour. Whereas the second hour secretion by islets exposed to mannoheptulose alone had returned to 74.5 per cent, the second hour secretion by islets exposed to both drugs was 28.5 per cent and, thus, not significantly different from 21.8 per cent (table 1), which was the level of secretion achieved with streptozotocin alone ($p > 0.2$).

DISCUSSION

The present experiments demonstrate that streptozotocin exerts a direct and immediate inhibitory effect on glucose-induced insulin secretion by isolated islets of Langerhans. This finding indicates that a direct action on the β cell, rather than a catecholamine response, is responsible for the early hyperglycemic phase of streptozotocin induced diabetes. Consistent with this view was the finding by Schein et al. of the early hyperglycemic response even in adrenalectomized animals.[10] Also suggesting a depression of secretion was the observation by Junod and coworkers of a lack of a rise in serum immunoreactive insulin during the early hyperglycemic phase.[7] In related works, Creutzfeldt and coworkers reported an impairment of secretion by islets removed one hour after streptozotocin or alloxan treatment in vivo,[19] while Taylor observed a reduction of insulin biosynthesis by isolated islets exposed to streptozotocin.[20]

Since streptozotocin is unstable under the conditions of our incubation,[1,21,22] it is quite likely that a break-

TABLE 6

Effect of mannoheptulose (MH) and nicotinamide (N) or mannoheptulose and streptozotocin (St) on glucose-induced secretion

First hour				Second hour			
Drugs and dose (mg./ml.)	Insulin output Mean ± S.E.M.	No. flasks	p	Drugs and dose (mg./ml.)	Insulin output Mean ± S.E.M.	No. flasks	p
MH (2.1)*	2.2 ± 1.1	(6)	< 0.001				
MH (2.1) + N (2.5)*	7.2 ± 1.8	(6)	< 0.001				
MH (2.1)	10.2 ± 3.0	(6)	< 0.001	nil	74.5 ± 6.2	(6)	< 0.01
MH (2.1) + St (0.5)	10.5 ± 1.6	(6)	< 0.001	nil	28.5 ± 5.2	(6)	< 0.001

*One ninety-minute incubation.

down product, rather than the drug itself is responsible for the observed effects. This is consistent with the suggestion of Schein that streptozotocin and related N-nitroso compounds exert their action via the release of diazoalkane.[23]

Our findings concerning nicotinamide suggest a direct and complete protection from streptozotocin's inhibition of glucose-induced secretion. Nicotinamide's protection appears specific for streptozotocin since mannoheptulose's inhibitory action was not blocked by nicotinamide. The ability of nicotinamide to protect secretion even when added to the medium only for the second hour of incubation (table 5) suggests that no permanent cytotoxic damage had been caused by streptozotocin over the first hour incubation. The inability of nicotinic acid to protect secretion might be due to its inefficacy, compared to nicotinamide, as a precursor of NAD.[24]

A number of analogies can be found between the effect of streptozotocin upon insulin secretion in the present study and its ability to cause permanent diabetes as reported in the literature. First, streptozotocin causes an irreversible hyperglycemia in vivo and exerts a marked inhibition of glucose-induced secretion in vitro. Nicotinamide protects from both these effects, but nicotinic acid does not. In both systems, in vivo using hyperglycemia as an index and in vitro using glucose-induced secretion as an index, nicotinamide's protective action is still marked even when administered one hour after streptozotocin.

The similar patterns listed above strongly suggest that the same biochemical lesion is responsible for both the impairment of insulin secretion and the occurrence of β cell destruction. The demonstration in the present study of the protective action of nicotinamide at the site of the islet cells indicates that the NAD level of the β cell may be critical for the maintenance of normal physiology. One working hypothesis is that upon entry into the β cell, streptozotocin leads first to an impairment of insulin secretion and then to necrosis. Indeed, Stauffacher et al.[25] have concluded from experiments with agents known to increase available NAD and NADP, that the early hyperglycemic phase may be the first direct response to streptozotocin. In work reported during this writing, Hinz and coworkers[26] found that streptozotocin depressed the NAD content of isolated rat islets during the first two hours of incubation.

All these observations support the idea that the level of NAD in the β cell may be crucial for normal function. Also consistent with this hypothesis is our finding of a significant stimulation of glucose-induced secretion by nicotinamide. A similar finding for NADH, NADP and NADPH has been reported by Watkins et al.[27] using toadfish islet tissue.

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Influence of Age and Strain on the Response of Rats to Dietary Fructose and Sucrose

Fructose added to the drinking water has been found to increase serum and liver triglyceride levels in mature but not in young rats. Dietary sucrose has been found to decrease the oxidation of glucose and its incorporation into fat by liver slices of rats from some strains but not in others. Strain differences have also been observed with regard to the effect of dietary sucrose on hepatic glucose-6-phosphatase activity. These experiments demonstrate the need to consider age and strain in studies on the metabolic effects of dietary carbohydrates in rats.

Since the initial reports implicating dietary sucrose as an important contributing factor to the development of cardiovascular disease (J. Yudkin, Lancet 1:645, 1956; 2:4, 1964) several investigators have studied the effect of various dietary carbohydrates on circulating lipid levels. These studies have been reviewed periodically on these pages (Nutr. Rev. 23:231 and 292, 1965; 24:35 and 65, 1966; 25:78 and 102, 1967). Recently, experimentation with animals has been initiated in an attempt to define the mechanisms by which sucrose, and more specifically its fructose moiety, influences circulating lipid levels. Also, studies have been conducted to determine the effects of sucrose, fructose, or both on intermediary metabolism.

Two reports have appeared which illustrate some factors that must be considered in such investigations. P. Hill (Lipids 5: 621, 1970) has demonstrated that age will significantly influence the lipemic response to sucrose ingestion in rats. A. E. Bender, K. B. Damji, and C. G. R. Yapa (Biochem. J. 119: 351, 1970) have shown that the effect of dietary sucrose on hepatic glucose utilization and glucose-6-phosphatase activity differs in rats of various strains.

The experiments reported by Hill were conducted to determine whether the increased serum triglyceride levels induced by fructose ingestion would be maintained and to determine the effect of fructose ingestion on hepatic cholesterol synthesis. Rats of two ages were used, young rats weighing 120 gm. and mature albino rats of both sexes weighing 180 to 250 gm. A commercial laboratory food was fed ad libitum and fructose, when given, was provided in the drinking water at a level of 10 per cent (w/w). The experiments were continued for twenty-one days. Providing 10 per cent fructose in the drinking water did not influence circulating triglyceride levels in young rats but produced a significant and sustained hypertriglyceridemia in mature animals. Serum phospholipid levels were significantly higher in both young and mature rats given fructose. Liver triglyceride levels were also increased in mature but not in young rats given fructose. The accumulation of triglyceride in livers of mature rats given fructose in their drinking water was not seen in fasted animals.

Fructose administration was not found to influence hepatic triglyceride synthesis from H-3-glycerol or acetate-2-C-14. However, hepatic cholesterol synthesis was decreased in rats fed fructose. The results of these studies are difficult to interpret, and many aspects of these experiments could be criticized; for example, the use of a purified diet would have provided better dietary control and enabled the quantitation of sucrose intake. It is also unfortunate that the strain of rats used was not specified. Nonetheless, the observation that fructose ingestion was hyperlipemic in mature but not in young animals appears to be particularly significant.

Bender et al. (Biochem. J. 119:351, 1970) fed male 28-day-old rats diets containing either 60 per cent sucrose or starch, and 24 per cent casein, 8 per cent peanut oil, and adequate amounts of vitamins and minerals. These diets were fed for twenty-five days, after which the animals were sacrificed. The ability of liver slices from these rats to oxidize glucose-C-14 or to incorporate it into fatty acids was determined. Also,

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