Progressive Damage of Cultured Pancreatic Islets After Single Early Exposure to Streptozocin

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SUMMARY

To examine effects of streptozocin (STZ) on pancreatic islets in the absence of a functioning immune system, we examined isolated rat islets cultured for 96 h after a single 1-h exposure to STZ in vitro. In addition to an immediate and sustained suppression of insulin secretion, STZ also induced a progressive decline in insulin content per islet as well as in total islet tissue mass, characterized by a decrease in both islet number and volume. Viability studies show that STZ-induced cell death was also progressive and was not commensurate with loss of secretory function. Furthermore, media-transfer experiments demonstrate that decline of tissue mass is not due to accumulation of metabolite or degradation products in the media. After 96 h in culture, untreated islets showed a marked insulinogenic capacity that was inhibited more than fourfold by the initial STZ treatment. Progressive loss of glucagon content per islet suggests that STZ causes disruption of islet morphological integrity. These progressive sequelae observed in vitro indicate that several aspects of the time-delayed attack on the β-cell by STZ are independent of a functioning immune system. DIABETES 1986; 35:1027–33.

The *N*-methylnitrosourea derivative, streptozocin (STZ), is a potent diabetogenic agent with a high degree of β-cell toxicity both in vivo[1,2] and in vitro.[3,4] Suppression of insulin secretion and biosynthesis by STZ is rapid and sustained, occurring within 1 h of exposure; in vivo it is followed by progressive necrosis and death of the β-cell in 1–3 days, resulting in permanent hyperglycemia (reviewed in ref. 5). Depending on species, dose, and the methods of in vivo administration, varying degrees of lymphocytic infiltration of the islets are induced by STZ, and an immune response is thought to be at least partly responsible for some of the observed progressive cell damage.[6] Hyperglycemia induced by STZ can be prevented or delayed by several disparate agents, e.g., antilymphocyte serum,[6] nicotinamide,[1] and ethanol,[1] only some of which are considered to act at the level of the immune system. Using a single low dose of STZ, Riley et al.[7] brought on a protracted gradual development of severe diabetes in mice over a period of weeks with only occasional observations of insulitis. Thus, it appears that in vivo an initial transient exposure to STZ can induce a progressively increasing hyperglycemia that may develop to some degree by nonimmune mechanisms. In our study, we examined the action of STZ on rat islets of Langerhans cultured over several days to explore the progression and permanence of the STZ insult over time in the absence of any contributing immune system.

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MATERIALS AND METHODS

Isolation and treatment of islets with streptozocin. Collagenase-dispersed islets were isolated from fed Long-Evans rats (Simonsen, Gilroy, CA) by the method of Lacy and Kostianovsky,[8] with modifications previously described.[9]

Equal numbers of islets (35–70/experiment) were randomly aliquoted to 35 × 10-mm plastic Petri dishes and washed twice in Krebs-Ringer bicarbonate medium (KRB), pH 7.4, with 2 mM glucose. This and all subsequent washes throughout the experiment were performed by transferring the islets in ~40 μl buffer to a dish containing 3 ml fresh buffer. Islets were incubated for 1 h at 37°C under a 95% O_2-5% CO_2 atmosphere in 2 ml of KRB (control), KRB plus 0.5 mg/ml STZ (low dose), or KRB plus 1.5 mg/ml STZ (high dose). The STZ (courtesy of Upjohn, Kalamazoo, MI) was dissolved in medium immediately before use. At the end of 1 h, islets were washed three times with KRB and then divided for either assessment of islet function or for 96-h culture.

Islet function. Initially, islet function was tested by measuring insulin responsiveness to glucose in a defined system; 10 islets from each group described above were incubated in duplicate or triplicate in 1 ml KRB with 2 or 20 mM glucose

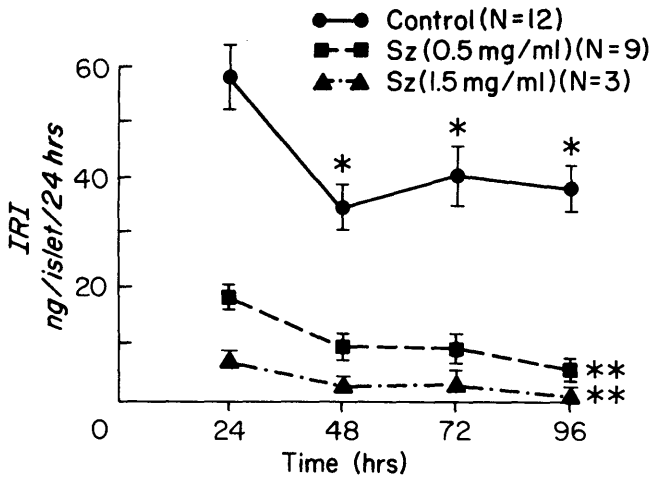

FIG. 1. Effect of 1-h exposure to low or high streptozocin (STZ, Sz) on chronic insulin secretion in islets cultured in RPMI-1640 up to 96 h. Total insulin accumulated in media was measured every 24 h. *Significant differences in insulin accumulation in control islets vs. 24-h control ($P < .01$); **insulin accumulation in STZ-treated islets is significantly different from insulin accumulation in untreated control islets at corresponding time points ($P < .01$).

under a 95% O_2-5% CO_2 atmosphere for 1 h. Aliquots of this incubation medium were diluted in 0.01 M Na_2HPO_4, 0.1% bovine serum albumin (BSA), and 0.025% thimerosal and stored at −20°C until immunoassay for insulin; the islets were extracted overnight in 300 μl acid:ethanol with benzamidine, diluted as above, and immunoassayed for insulin.

Islet culture. The remaining islets were washed five times in sterile culture medium [RPMI-1640 (Gibco, Santa Clara, CA), with 25 mM Hepes, 2 μ/ml Pen-Strep, 2.0 μg/ml Fungizone (Gibco), 10% heat-inactivated fetal calf serum (FCS), and 20 mM glucose] and cultured for 96 h total, with the start of culture considered to be 0 h. The media were changed every 24 h after quintuple washes, and each 24-h sample was immunoassayed for insulin. At the end of 96 h, the remaining islets were challenged with glucose in batches of 10 as described above and media collected for insulin radioimmunoassay; islets were also extracted in acid:ethanol for insulin and glucagon immunoassay with a solid-phase system and an automatic pipetting station (Micromedic Systems, Horsham, PA).[10]

Islet number and volume. In separate batches of islets cultured after exposure to STZ, the number of islets in each culture dish was counted at 0, 48, and 96 h. Islet volume was followed during the course of the incubation by a dissecting microscope with stage and ocular micrometers to measure long and short axes. If islets are assumed to be roughly prolate ellipses, islet volume can be calculated by the formula $V = 4/3\,ab^2$, where a = major semiaxis and b = minor semiaxis. Islets used in these measurements were not used for further measurements of insulin responsiveness to glucose challenge or of islet hormone content.

Cell viability. To assess changes in cell viability over time, trypan blue exclusion was measured in aliquots of islets cultured in RPMI-1640 for 0, 24, and 48 h after exposure to 0.5 mg/ml STZ. Islets, washed three times with Ca-free KRB, were incubated at 30°C for 15 min in STV dissociation medium (normal saline with .025% trypsin, .01% EDTA, and 4 mg/dl DNase) with occasional trituration with a Pasteur pipette. After two washes with cold KRB, dissociated cells were dispersed in 0.2% trypan blue/KRB, and the percentage of viable cells was counted in a hematocytometer.

Media-transfer experiments. To ensure that culture media did not accumulate stimulatory or inhibitory growth factors, media-transfer experiments were performed for 96 h. These transfer experiments were also designed to test the assumption that the STZ added to the media the day before had been degraded in a relatively short time and was no longer potent in the 24-h-old media.[11] Freshly isolated islets were divided into four groups, one that was treated with 0.5 mg/ml STZ (group S) and three with KRB (groups A, B, and C) for 1 h, as described above, and then incubated overnight in standard culture medium. Untreated group A islets were exposed to fresh media daily, whereas untreated group B islets were cultured in fresh media for the first 24 h and then in sterile filtered standard media used by group A islets for the previous 24 h. Group S islets received fresh culture media

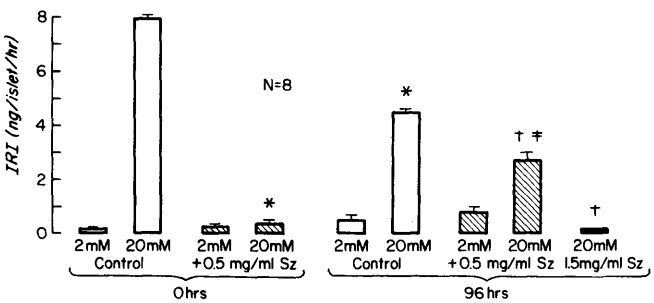

FIG. 2. Effect of STZ on insulin response to acute glucose stimulation. Islets were challenged with 20 mM glucose, 1 mM IBMX just after STZ treatment (0 h) and 96 h after STZ treatment. *Acute insulin response is significantly different from that in challenged control islets at 0 h ($P < .01$); †acute insulin response is significantly different in STZ-treated vs. control islets at 96 h ($P < .05$); ‡acute insulin response in STZ-treated islets at 96 h is significantly different from that in STZ-treated islets at 0 h ($P < .05$).

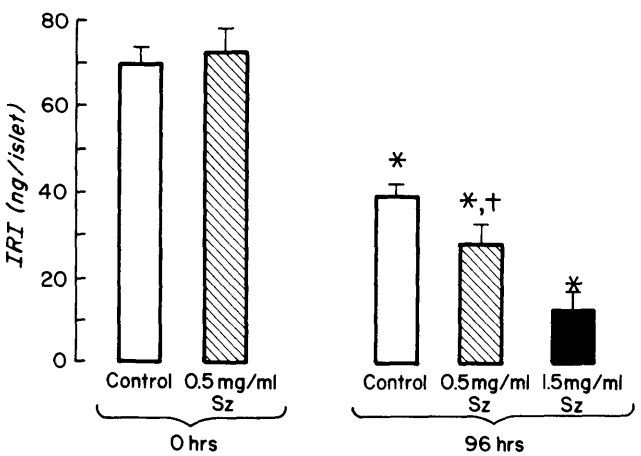

FIG. 3. Effect of STZ on insulin content of islets just after exposure (0 h) and in islets cultured for 96 h after 0.5 mg/ml STZ ($N = 7$) or 1.5 mg/ml STZ ($N = 3$). 0-h values (62.0 ± 17.1 ng/islet) at high STZ dose not included in figure for graphic simplicity. *96-h insulin content is significantly different from 0-h values ($P < .01$); †96-h insulin content of STZ-treated islets is significantly different from 96-h control islets ($P < .05$).

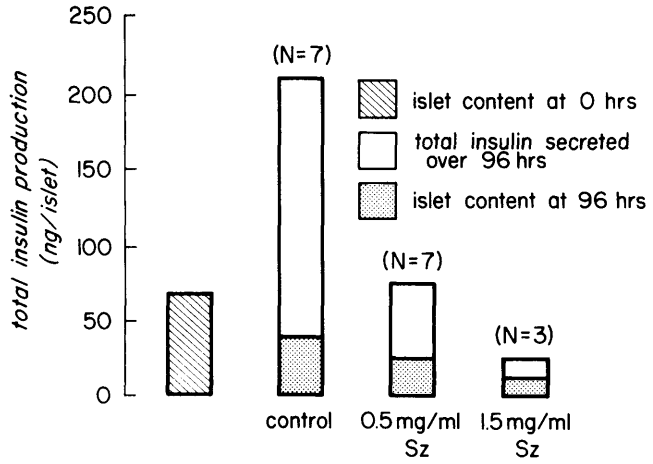

FIG. 4. Effect of STZ on total insulin production in islets cultured for 96 h after exposure to STZ, calculated by adding all secreted insulin and final islet content. Both low and high STZ significantly inhibit total insulin production ($P < .01$) vs. 96-h control.

daily, whereas untreated group C islets were cultured in fresh culture media for the first 24 h and then in the sterile filtered culture media used by the STZ-treated islets (S) for the previous 24-h period. This procedure was repeated daily and islet mass was measured at each media change until the end of the 96-h culture period.

Statistical analyses. Multiple comparisons were analyzed by ANOVA and Newman-Keuls multiple-range tests.

RESULTS

During culture at 20 mM glucose, normal rat islets sustained insulin secretory function for 96 h and demonstrated insulin responsiveness to glucose stimulation throughout the course of the experiment (Figs. 1 and 2). Secretion from normal islets, measured as accumulation in the media (Fig. 1), was maximal at 24 h, declining ~40% by 48 h ($P < .01$), and remaining at this lower but still substantial plateau for the following 48 h. Islets treated with 0.5 mg/ml STZ showed a 68% inhibition of insulin secretion in the first 24 h ($P < .01$, vs. 24-h control), declining progressively over the 96-h incubation ($P < .01$, vs. 96-h control). High levels of STZ (1.5 mg/ml) inhibited secretion more severely, by 85%, at the end of the first day ($P < .01$, vs. 24-h control) and to essentially nondetectable levels of insulin secretion by 96 h (Fig. 1).

Figure 2 demonstrates that at 0 h, the response of normal islets to a glucose challenge in a defined system was a brisk 8.0 ng · islet^{-1} · h^{-1}, which was reduced 50% by 96 h ($P < .01$), similar to the decrease in chronic insulin release over 96 h seen in Fig. 1. Inhibition of insulin secretion by STZ occurred rapidly (Fig. 2); in the islet-function test of the 0-h group performed at 1 h after STZ exposure, low doses of STZ completely suppressed insulin secretion. By 96 h, the residual STZ-treated islets escaped this inhibition somewhat, since the response to glucose challenge (20 mM) was now significantly different from unchallenged (2 mM) STZ-treated islets ($P < .05$). However, the response was significantly less than that of the glucose-challenged control islets ($P < .05$). The high-dose STZ completely suppressed the insulin response to glucose at 0 h, and there was no detectable recovery of function at 96 h (Fig. 2). Insulin content per islet declined in all islet preparations (Fig. 3), declining ~50% in control islets by 96 h ($P < .01$, vs. 0-h control) and by 60% in islets treated with low doses of STZ ($P < .05$, vs. 96-h control). The insulin content of islets treated with high doses of STZ was the most depressed, measuring only 21% at the end of the culture period.

The total amount of insulin produced by normal and STZ-treated islets over the duration of the culture period was calculated by summing total secreted insulin and total tissue insulin content in each experimental group of islets remaining at the end of the culture period, as shown in Fig. 4. Despite small losses anticipated in the culture media during each 24-h period, total insulin increased in controls, reflecting active insulinogenesis. This was inhibited >65% by exposure to low-dose STZ and >85% by high-dose STZ, demonstrating a significant inhibition of islet-production capacity by STZ in a dose-dependent manner.

Figures 5–7 demonstrate the progressive effects of a single exposure to STZ on both islet number and volume. Control islet tissue showed an insignificant 5% decline in islet number (Fig. 5) and a similar modest decline in average islet volume from 8.6 to 7.2 × 10^3 mm^3 over 96 h (Fig. 6). In contrast, islets treated with low doses of STZ showed a progressive 10% loss in islet number and a progressive 60% loss in volume per islet to a final volume of only 3.6 × 10^3 mm^3 measurable by 96 h. Islets treated with high doses of STZ

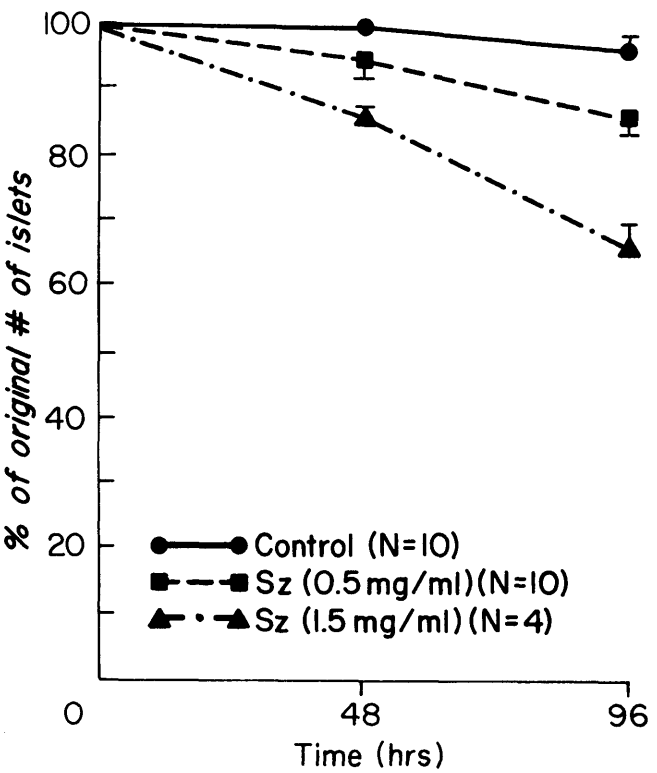

FIG. 5. Relative changes in islet number over 96 h in culture in control and STZ-treated islets. Islet numbers are represented as percentage of original number of islets in each experiment. Control islet values drop only moderately from 54.9 ± 5.04 islets at 0 h to 52.9 ± 4.46 by 96 h, whereas islets treated with low doses of STZ decline from 54.5 ± 5.11 at 0 h to 46.9 ± 3.93 islets by 96 h ($P < .05$), and islets treated with high doses of STZ drop from 46.3 ± 14.91 at 0 h to 31.0 ± 11.05 islets by 96 h ($P < .01$).

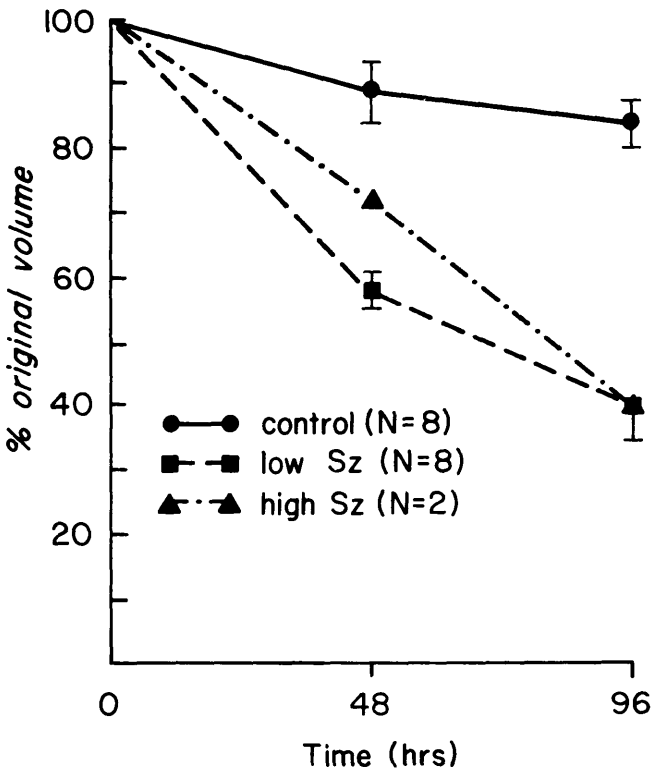

FIG. 6. Relative changes in islet volume over 96 h in culture in control and STZ-treated islets. Volume per islet, represented as percentage of original volume, changed little in control islets, from 8.6 ± .32 at 0 h to 7.2 ± .34 × 10^3 mm^3 by 96 h, but declined significantly in islets treated with low doses of STZ, from 9.01 ± .30 to 3.6 ± .48 × 10^3 mm^3 by 96 h ($P < .01$), and similarly in islets treated with high doses of STZ, from 6.2 × 10^3 mm^3 at 0 h to 2.5 × 10^3 mm^3 at 96 h.

showed an even greater loss in islet number (35%) and a 60% drop in average volume per islet, similar to that seen in the group treated with low doses of STZ (Figs. 5 and 6). Figure 7 demonstrates the continuing loss of total islet tissue (calculated as average volume × islet number) after an initial exposure to STZ.

The cell viability data (Table 1) show that the percentage of viable cells per islet was slightly less in the STZ-treated group than in the control groups. However, the percentage of viable cells per islet was high and did not decrease over time in either group; control islets always contained at least 95% viable cells at 0, 24, and 48 h, whereas STZ-treated islets had >80% viable cells per islet at all time points.

During each 24-h culture period, despite the replenishment with fresh media, increasingly cloudy media as well as tissue debris became noticeable over the course of the experiment in both STZ-treated groups, particularly in the islets treated with high doses of STZ. In contrast, media from control islets was clear at each 24-h period, and no tissue debris was apparent. Under the dissecting microscope, STZ-treated islets were more irregularly shaped and ragged, consistent with the greater amount of debris observed.

Normal islets cultured in media transferred from STZ-treated islets showed a small (8–15%) loss in average volume, similar to that seen in normal islets cultured in fresh media (Fig. 8 vs. Fig. 5), indicating that the progressive damage to islet tissue after STZ treatment is not due to increasing toxic-substance activity. Islet glucagon content, measured as an index of peripheral cell loss, was decreased 96 h after STZ exposure in a dose-related manner, whereas control islet levels were unchanged (Fig. 9).

DISCUSSION

In our study, we examined the change in insulin secretion from isolated rat pancreatic islets cultured for 96 h after an initial 1-h exposure to the β-cell toxin STZ. Although the mechanisms of STZ damage to the β-cell are not well understood, the rapid inhibition of insulin biosynthesis and secretion in vivo is well documented.[1,5,12] In vitro a single short exposure to STZ is sufficient to rapidly suppress islet function for at least several hours in isolated rat islets[4,13,14] and several days in β-cell monolayer culture.[15] We demonstrated that a single short exposure to STZ has a complex temporal effect on islet function, with an immediate and sustained suppression of insulin secretion as well as more gradual changes indicative of progressive cell death seen over several days.

Initial suppression of insulin secretion occurred within 1 h of STZ exposure and became progressively worse during the 96-h culture. Furthermore, STZ-induced reduction in islet tissue mass was also progressive over time, decreasing as rapidly during the last 2 days of culture as during the first 2 days. The rationale for STZ's extended biologic potency is uncertain, because the toxin is rapidly degraded at physiologic pH,[16] with a half-life of ~5 min.[11] Possibly by virtue of

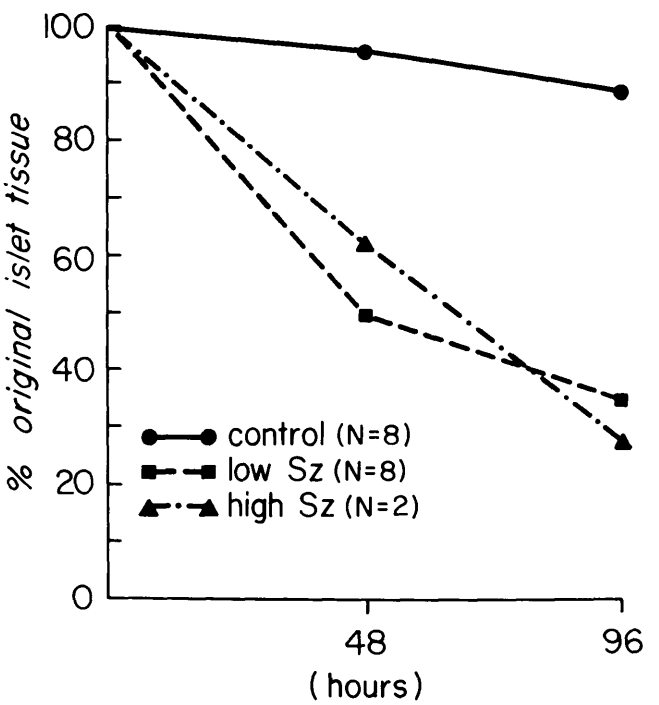

FIG. 7. Relative changes in total islet tissue over 96 h in culture in control and STZ-treated islets. Islet mass (volume · $islet^{-1}$ · islet number), represented as the percentage of starting value, declined from 342 × 10^3 mm^3 at 0 h to 287 × 10^3 mm^3 at 96 h (NS) in control islets and declined significantly in islets treated with low doses of STZ, from 358 × 10^3 mm^3 at 0 h to 131 × 10^3 mm^3 by 96 h ($P < .01$), as well as in islets treated with high doses of STZ, from 287 × 10^3 mm^3 at 0 h to 77.5 × 10^3 mm^3 by 96 h ($P < .01$).

TABLE 1
Percentage of viable cells per islet

Treatment	Time (h)		
	0	24	48
Control	94.7 ± 2.5	96.9 ± 0.5	97.8 ± 0.1
STZ	83.3 ± 1.2*	91.4 ± 1.6*†	80.7 ± 1.5*

Percentage ± SE of viable cells, measured by trypan blue exclusion, in islets incubated in RPMI-1640 for 0, 24, or 48 h after initial 1-h exposure to 0.5 mg/ml STZ.
*$P < .05$, STZ group vs. respective control group at each time point ($N = 3$).
†$P < .05$, 0 vs. 48 h, STZ group ($N = 3$).

its glucose moiety, STZ has a high degree of affinity for the β-cells in the pancreas, where STZ concentrations exceed blood levels within minutes of intravenous administration.[17] Streptozocin or a toxic metabolite such as *N*-methylnitrosourea could be actively sequestered by the β-cell and have an extended biological action at the cellular level. However, in the absence of more definitive studies of intracellular localization of labeled STZ, the question of internalization of STZ and extended biological potency remains unclarified.

Alternatively, STZ may provoke a variety of physiologic insults to the β-cell that singly or in combination would account for continued suppression of insulin synthesis and secretion as well as declining cell function and eventual cell death after a single short exposure to the cytotoxin. Orci et al.[18] have shown morphological changes of β-cell membrane-associated particles within 15 min of STZ exposure, suggesting that membrane damage may be an early event in STZ action. Dean and Matthews[19] found that, although STZ did not alter islet β-cell membrane potential within 60 min, it did suppress propagation of action potentials induced by glucose and a variety of secretagogues. These data suggest that STZ is altering transmembrane ion fluxes, which are known to be coupled with glucose-stimulated insulin secretion (reviewed in ref. 12) and to participate in early loss of responsiveness to glucose stimulation. As further evidence of STZ damage to membrane integrity, dispersed islet cells when preloaded with ^{51}Cr leak significantly more radioisotope within 4 h after STZ exposure.[20]

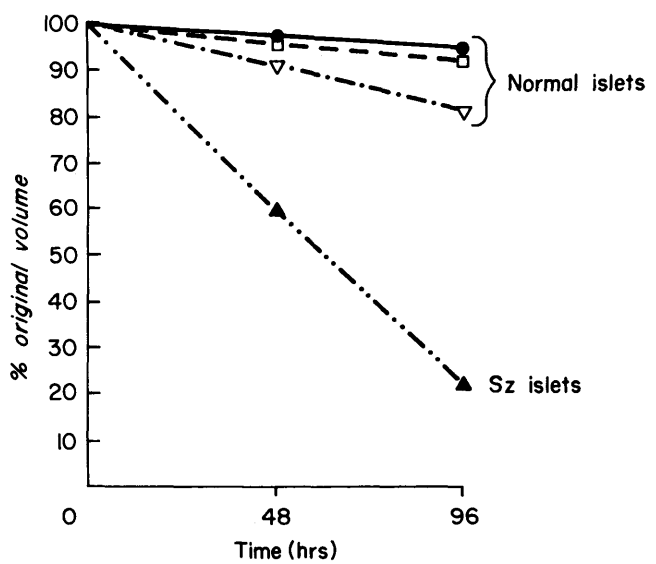

FIG. 8. Effect of media on changes in islet volume in islets cultured for 96 h after exposure to STZ. Normal islets from group A (●) cultured in fresh RPMI daily for 96 h declined slightly from 7.46 to 7.02 mm³; normal islets from group B (□) cultured in fresh RPMI for 24 h and then in group A's media from the previous day declined from 8.44 to 6.85 mm³; normal islets from group C (△) cultured in fresh RPMI for 24 h and then in media from STZ-treated islets from previous day declined from 7.15 to 6.53 mm³; islets treated for 1 h with STZ (0.5 mg/ml, △) and then cultured in fresh RPMI daily for 96 h declined from 7.15 to 1.72 mm³. $N = 2$ for each variable.

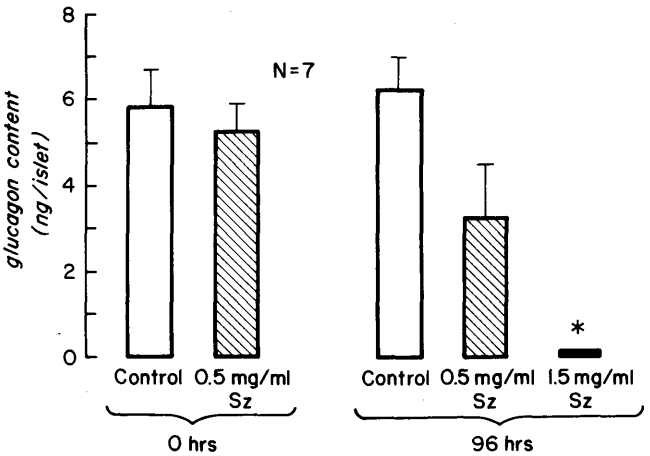

FIG. 9. Effect of STZ on glucagon content of islets just after exposure to STZ (0 h) and in islets cultured for 96 h after STZ. *Glucagon levels are significantly different in high-STZ group vs. control or low-STZ group ($P < .01$). Glucagon in low-STZ islets is depressed but not significantly vs. control islets.

Considerable evidence suggests that STZ inhibition of insulin secretion is mediated by STZ alkylation of DNA, which causes DNA strand breakage and subsequent activation of the nuclear repair enzyme poly(ADP-ribose) synthetase. Protection against effects of STZ on insulin secretion and β-cell membrane by pyridine nucleotides and other inhibitors of poly(ADP) ribosylation is well documented in vivo[1] as well as in isolated islet preparations.[13,18,19,21–23] Using primary cultures of rat β-cells, Wilson et al.[15] showed that, if presented at or before exposure to STZ, inhibitors of poly(ADP) ribosylation also protect insulin secretion throughout a 96-h study. Poly(ADP-ribose) synthetase consumes cellular stores of NAD by transferring the ADP-ribose moiety of NAD to various nuclear proteins, with a resulting liberation of nicotinamide (reviewed in ref. 13). As a result of the increased poly(ADP) ribosylation activity, a severe 50–90% depression of islet levels of NAD is apparent as early as 15 min after STZ exposure[13,21–24] and lasts for at least 5 h.[24] The extent of STZ-induced depression of islet NAD levels is not known after 5 h,[24] although liver NAD, depressed for 24 h, recovers to control values by 48 h after STZ exposure.[25] The depletion in NAD concentrations may be responsible for a fall in ATP stores, which has been observed after STZ exposure,[4,14] and could account for the rapid inhibition of insulin secretion. In human lymphocytes, correlation between cell concentrations of ATP and NAD has been established.[26] In our recent studies, we found that islet NAD stores may not permanently be

suppressed by STZ, whereas inhibition of insulin secretion persists (J. L. Bolaffi, S. Nagamatsu, S. Harris, and G. M. Grodsky, unpublished observations). Thus, a different or additional action of STZ should be considered.

Recently, induction of superoxide dismutase (SOD) activity by the superoxide radicals has been linked temporally to poly(ADP-ribose) synthetase activity in lung tissue exposed to O_3 over several days.[27] However, several investigators have demonstrated that quenching of the superoxide radical by SOD offers no protection against STZ.[13,15,28]

Over time, the effect of STZ on glucose-stimulated insulin secretion has an interesting dose-related component. By 96 h, control islet secretion to high glucose diminishes by ~50% compared with 0 h, although the secretory response per islet is still a ninefold increase over basal secretion, indicating that culture conditions maintain a substantial secretory function (Figs. 1 and 2). This attenuated basal secretory rate of control islets is an expression of islet desensitization, which we have shown begins after 3–4 h of continued glucose stimulation[29] and may reflect a common phenomenon of secretory cells.[30,31] Islets treated with 0.5 mg/ml STZ show a modest recovery by 96 h (0.3 vs. 2.7 ng/islet, 0 vs. 96 h), whereas islets treated with 1.5 mg/ml show no recovery (0.1 vs. 0.1 ng/ml). Because the amount of insulin secreted is calculated as nanograms hormone per islet, the partial recovery of islet secretory function suggests that, at least at low STZ concentrations, some β-cells have escaped the initial inhibition of STZ or perhaps are capable of repairing the initial lesion to some degree.

In addition to the loss of β-cell secretory activity, we also found loss of islet insulin biosynthesis capacity (measured as total insulin recovered in the system), some of which is due to long-term culture and some of which is clearly due to the initial STZ exposure. In untreated control islets, a production capacity of >200 ng insulin/islet over 4 days indicates a high degree of viable biosynthetic machinery throughout the culture period. In contrast, STZ induces a dose- and time-dependent loss of insulin synthetic capacity (Fig. 4), confirming and expanding pulse-chase experiments of others.[4,13,32] Both the amount of insulin secreted per day and the insulin content per islet are significantly depressed by STZ, with a sevenfold reduction of total production capacity in the high-STZ group.

In untreated islets, the loss of total islet tissue itself is minimal, with almost 90% of control islet tissue remaining at the end of 4 days, even though islets had been handled extensively during daily washes and transfers. Thus, although control islets show some decline during culture, most islet tissue and function persist throughout the extent of the culture period. The significant loss of islet tissue itself caused by STZ increases over time (Fig. 7); this is also readily observed during daily media changes by macroscopic bits of tissue and debris that cloud the media, especially in the high-STZ culture.

We also considered that STZ may have caused instant cell death and that the progressive loss in islet tissue observed may have simply been the result of progressive decomposition and disruption of the cytoskeletal structure. However, this was not the case, as demonstrated by the cell viability studies. Total loss of islet tissue over 96 h averaged 60%. If STZ damage to the β-cell was maximum at the initial attack, the cells killed in the 1st h of STZ treatment would be only partially sloughed and lost by 24 h, and a maximal number of dead cells per islet would be expected initially, followed by a declining percentage of dead cells that should approach the levels seen in control islets with time. Instead, we found a low and rather constant 15–20% level of dead cells in the STZ group, although always significantly greater than that seen in the control group, indicating that cell death progressively continued for 2 days after the initial STZ insult. The small increase in percentage of viable cells at 24 h was observed in both STZ and control groups and may reflect heightened islet sensitivity soon after collagenase dispersion.

One other aspect of the cell viability studies emphasizes that β-cell secretory activity, although extremely sensitive to STZ attack, should not be equated with cell death. Thus, STZ inhibited chronic insulin secretion by 68% and completely suppressed glucose-stimulated insulin secretion by the first 24 h, although the islets never contained more than 20% dead cells.

Autoimmune destruction of pancreatic islets, triggered by low doses of STZ,[6] has been postulated to be a basic mechanism of STZ-induced islet necrosis. However, our data showing progressive damage in islets cultured over 4 days, and thus in the absence of a functioning immune system, indicate that continuing damage after brief exposure to STZ occurs directly at the β-cell level.

The media-transfer experiments (Fig. 8) show that culture media from STZ or control islets did not affect islet volume and insulin secretion, indicating that toxic or degradative compounds liberated during islet disintegration did not cause the progressive tissue loss and/or reduced media insulin content.

The loss of glucagon content per islet after 96 h culture, although modest, may arise from STZ-induced β-cell necrosis. In vivo, STZ has little effect on the islet α-cell[5]; because β-cells comprise ~80% of islet mass, the loss of glucagon per islet in vitro suggests that the loss of α-cells results from general disorganization after massive β-cell destruction.

In summary, we have demonstrated that for 96 h in culture, a single short exposure to STZ results in a rapid suppression of islet secretory function and insulinogenic capacity and loss of tissue mass, which progresses throughout the culture period. Such progressive sequelae in vitro indicate that time-delayed attack on the β-cell by STZ can be independent of autoimmune responses.

ACKNOWLEDGMENTS

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