Pyridine Nucleotide Depletion in Pancreatic Islets Associated with Streptozotocin-induced Diabetes

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SUMMARY

An investigation of NAD-NADH concentrations in the pancreatic islets of rats following diabetogenic doses (65 mg./kg.) of streptozotocin (SZ), given singly or in combination with nicotinamide or nicotinic acid, is reported. After a given treatment of the animals, the pancreases were surgically removed. The concentrations of NAD and NADH were determined on islets isolated from freeze-dried sections of the pancreas. In the pancreatic islets of untreated control animals, the mean ± S.E. concentrations of NAD and NADH were 1,122 ± 57 and 129 ± 28 mμmoles/gm. dry tissue, respectively. The NAD content of the islets decreased to 27 per cent of the control value one hour and to 12 per cent five hours after administration of SZ. NADH concentration five hours after SZ was significantly reduced from the control value. When nicotinamide was given thirty minutes before SZ, the depletion of NAD associated with SZ administration was prevented. When nicotinamide was given one hour after SZ, the depressed NAD concentration observed at this time was subsequently raised. In contrast, nicotinic acid given thirty minutes prior to SZ did not prevent depletion of NAD. The data suggest that streptozotocin-induced beta-cell deterioration may be etiologically related to depletion of cellular NAD. DIABETES *21*:789-93, July, 1972.

Streptozotocin, N (methyl, nitroso)—N′ (2 glucose) urea, induces diabetes in many species of animals by destroying the beta cells of pancreatic islets without apparent injury to the alpha cells.[1,2] The diabetogenic effect of streptozotocin can be antagonized by nicotinamide.[3-8] Both nicotinamide and nicotinic acid are precursors of nicotinamide adenine dinucleotide (NAD).[9,10] However, unlike the amide, the acid is incapable of preventing the diabetes induced by streptozotocin.[3,5,6,8] Administration of streptozotocin results in depletion of the NAD content of the liver, and nicotinamide is capable of preventing such depletion.[3,4] Thus, it was reasoned that, if streptozotocin and nicotinamide were to exert the actions on the beta cells of pancreatic islets as they do on the liver, the mechanism of streptozotocin-induced diabetes and that of the protective effect of nicotinamide might be related to the effects of these compounds on the cellular NAD content. In an attempt to elucidate such a mechanism, studies were made of the content of NAD and its reduced form, NADH, in pancreatic islets after diabetogenic doses of streptozotocin, injected alone or in temporal association with nicotinamide or nicotinic acid.

MATERIALS AND METHODS

Male Sprague-Dawley rats, weighing 400 to 500 gm., were used. Streptozotocin,* in a dosage of 65 mg./kg. body weight, was dissolved immediately before injection in 1 ml. of 0.9 per cent saline acidified to pH 4.5 with 0.05 M citric acid and injected via a tail vein. Nicotinamide was dissolved in 1 ml. of normal saline and administered intraperitoneally. Nicotinic acid was dissolved in approximately 1 ml. of 1.0 N sodium hydroxide and the pH of the solution was adjusted to 7 in a final volume of 1 ml. The animals were given free access to food throughout the experiment. Thirty-five animals were divided equally into seven groups. Group 1 served as the normal control; no treatment was given before the pancreas was removed surgically. Group 2 received an intravenous injection of 1 ml. of 0.9 per cent saline acidified to pH 4.5 with 0.05 M citric acid. Group 3 was injected with streptozotocin. Groups 4 and 5 re-

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ceived 250 mg./kg. of nicotinamide and nicotinic acid respectively, one-half hour prior to streptozotocin. In groups 2 to 5, the pancreas was removed one hour following saline or streptozotocin injection. Group 6 was given a dose of streptozotocin followed by an injection of nicotinamide (500 mg./kg.) one hour later. Group 7 received only streptozotocin. In groups 6 and 7 the pancreas was removed five hours after streptozotocin administration. All injections and laparotomies were performed while the animals were under ether anesthesia.

Each pancreas was placed in liquid nitrogen immediately after removal. The tissue then was cut at a thickness of 75 microns in a cryostat previously cooled to —20° C. and the sections were dried under high vacuum at —20° C. for thirty-six hours.

The pancreatic islets were isolated from the freeze-dried sections by microdissection at room temperature. Under transmitted light, the islets appeared as slightly translucent, yellowish to pinkish masses of cells with round, oval or elongated contours. Under weak reflected light, they appeared slightly darker than the surrounding tissue which was white. Capillaries containing erythrocytes were observed frequently within the islets. The isolated islets were weighed with a quartz fiber balance.[11] The weight of islet tissue used for assay varied from 5 to 8 μg.

After weighing, the islets were placed in a dry conical centrifuge tube and thoroughly homogenized with a glass rod. Ice-cold 0.02 N NaOH solution was added to extract the pyridine nucleotides;[12] an amount of 10 μL. per μg. of islets was used. With the tube immersed in an ice bath the suspension was stirred with the glass rod for a few minutes. An aliquot of 10 μL. was pipetted into each of four tubes immersed in an ice bath. The NaOH extract contained both NAD and NADH.[12] To determine NADH, 10 μL. of 0.02 N NaOH was added to the first tube, which then was heated at 60° C. for fifteen minutes to destroy NAD. To determine NAD, 10 μL. of 0.1 N HCl was added to the second tube, which was heated at 50° C. for fifteen minutes to inactivate NADH. To determine the sum of NAD and NADH, 10 μL. of ice-cold 0.02 N NaOH was added to the third tube. The fourth tube, serving as a blank, was heated at 60° C. for fifteen minutes; thereafter 10 μL. of 0.1 N HCl was added and the tube was heated again at 50° C. for fifteen minutes. Following these treatments, the tubes were processed further according to the enzymatic cycling method of Lowry et al.[13] for the determination of the pyridine nucleotides. The determination was begun within one day following completion of the freeze-drying of the pancreatic sections. There was no interference with the measurement of 0.4 to 1.6 μμmoles of NAD or NADH by the presence of 80,000 μμmoles of streptozotocin, nicotinamide or nicotinic acid in the sample.

The concentrations of pyridine nucleotides in normal rat liver have been well established.[14] In order to evaluate the reliability of the ultra-microtechnics used in this investigation, as well as to provide a reference of com-

TABLE 1

NAD and NADH content (mμmoles/gm. dry tissue) of rat pancreatic islets under different experimental conditions. Group 1 received no treatment; group 2 received acidified saline; group 3 was given streptozotocin (SZ) intravenously (65 mg./kg. in acidified saline); group 4 was injected intraperitoneally with 250 mg./kg. of nicotinamide (NM), and group 5 with equivalent amount of nicotinic acid, thirty minutes prior to SZ. In groups 2 through 5 the pancreas was removed one hour after saline or SZ injection. Group 6 was treated with NM (500 mg./kg.) one hour following SZ; group 7 received SZ only. In groups 6 and 7 the pancreas was removed five hours after SZ.

Group 1		Group 2		Group 3		Group 4	
NAD	NADH	NAD	NADH	NAD	NADH	NAD	NADH
1,060	41	885	175	357	28	1,200	60
1,152	196	1,125	103	278	78	1,016	68
1,176	147	1,371	95	300	69	785	156
941	93	766	77	260	125	1,020	163
1,280	170	975	83	317	154	1,073	106
1,122±57[a]	129±28[h]	1,024±105[b]	107±18	302±17[c]	91±22	1,019±67[d]	111±21

Group 5		Group 6		Group 7	
NAD	NADH	NAD	NADH	NAD	NADH
204	106	480	110	143	89
350	85	550	80	67	58
324	171	430	40	177	31
648	222	370	74	101	71
707	181	286	91	193	54
447±98[e]	153±25	423±45[f]	79±12	136±23[g]	61±10[i]

The figures on the bottom lines represent mean ± S.E.
$P < .001$ for a-c, b-c, a-g, c-g, a-e, f-g; $P < .01$ for b-e, d-e; $P < .05$ for c-f, h-i.

parison for the islet tissue, the NAD and NADH concentrations were determined on 5 to 8 μg. of freeze-dried samples of livers derived from six normal male rats. The tissue was processed in the manner similar to that described for the pancreas.

RESULTS

The NAD and NADH concentrations in the pancreatic islets of the seven groups of animals are shown in table 1. The time course of NAD and NADH concentrations following streptozotocin administration is shown in figure 1. There was no significant difference in the NAD and NADH concentrations between the nontreated (group 1) and the saline-treated (group 2) animals. However, in group 3, there was a striking decrease in the NAD content of the islets of Langerhans one hour after streptozotocin administration ($p < .001$). The concentration of NAD in the islets was decreased further at five hours (group 7). In contrast, the decrease in the NADH content of the islets was not striking in response to streptozotocin administration, although the mean five-hour value (group 7) was significantly lower ($p < .05$) than the zero-hour control value (group 1). In the rats given 250 mg./kg. of nicotinamide thirty minutes before streptozotocin administration (group 4), the NAD content of the islets did not significantly differ from that of the untreated normal (group 1) or the saline-treated animals (group 2). Five hours after streptozotocin administration, the NAD content in the islets of group 6, which was given nicotinamide one hour after streptozotocin, was significantly higher than that of group 7, which was not given nicotinamide ($p < .001$). When the NAD content in the islets of groups 3 and 6 were compared, the difference also was significant ($p < .05$). The animals pretreated with nicotinic acid (group 5) had a significantly lower ($p < .01$) NAD content in the islets than untreated (group 1), saline-treated (group 2), and nicotinamide pretreated (group 4) animals (figure 2). No significant difference in NAD content was observed between groups 3 and 5. The NADH concentrations in the islets did not differ significantly among the groups, except for the difference between groups 7 and 1.

The mean ± S.E. NAD and NADH concentrations in the liver of six normal male rats, determined on 5 to 8 μg. of freeze-dried tissue, were 521 ± 53 and 113 ±

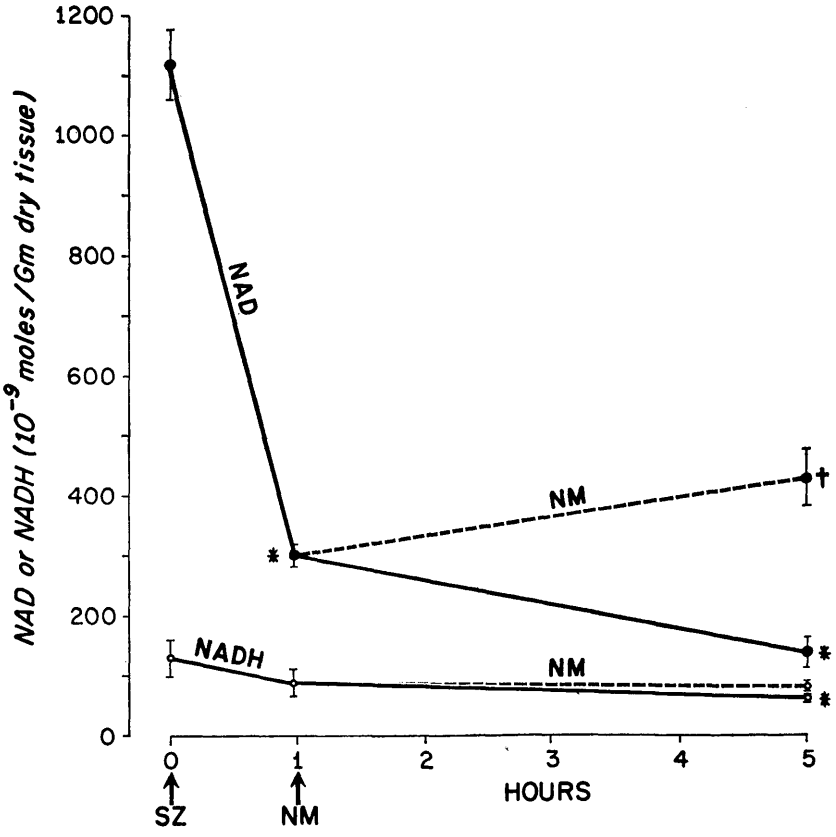

FIGURE 1

The time course of NAD and NADH content of rat pancreatic islets following intravenous administration of 65 mg./kg. of streptozotocin (SZ), singly (solid lines) or in combination with 500 mg./kg. of nicotinamide (NM) which was given one hour following SZ (broken lines). * = significantly lower than the value at zero hour (for NAD, $p < .001$; for NADH, $p < .05$). † = significantly higher than the values at five hours ($p < .001$) and at one hour ($p < .05$).

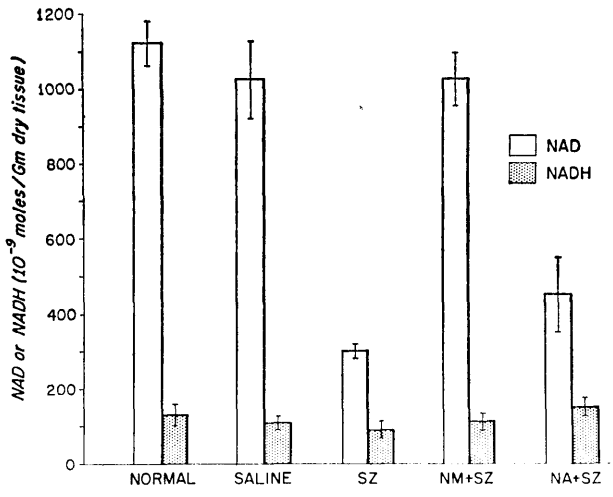

FIG. 2. Effect of streptozotocin (SZ), given alone or in combination with nicotinamide (NM) or nicotinic acid (NA), on the NAD and NADH content (mean ± S.E.) of rat pancreatic islets at one hour after administration. The normal group received no treatment. The saline group was given acidified saline intravenously; the SZ group was given 65 mg./kg. SZ in acidified saline intravenously; the NM + SZ and the NA + SZ groups were treated with nicotinamide and nicotinic acid respectively (250 mg./kg. intraperitoneally) thirty minutes prior to SZ.

12 mμmoles per gram wet weight respectively. In order to facilitate comparisons with the values reported in the literature, the NAD and NADH concentrations were expressed on the basis of wet weight of the tissue derived from dry weight by dividing the latter by a factor of 0.31. This factor was determined experimentally by subjecting fresh liver samples from six animals to the freeze-drying procedure. The mean ± S.E. water content was 68.8 ± 0.3 per cent of the weight of the fresh liver. The values for the concentrations of NAD-NADH are in agreement with those obtained with macromethods reported in the literature.[15]

DISCUSSION

The changes in the NAD and NADH concentrations in the islets under the different experimental conditions of the present study appear to have occurred mainly in the beta cells. Such an interpretation is based on the observations that the beta cells in the rat comprise approximately 80 per cent of the islet endocrine cells,[15] and that the alpha cells are resistant to streptozotocin.[2]

The damaging action of streptozotocin on the beta cell is fairly rapid. Nearly complete protection against diabetes occurs only when nicotinamide is given between thirty minutes prior to and ten minutes following the administration of streptozotocin. However, when nicotinamide is administered at times outside this interval, partial protection from the diabetogenic effect of streptozotocin is obtained.[7,8] The data presented in this study show that pretreatment at thirty minutes with nicotinamide results in virtually complete preservation of islet cell NAD and that post-treatment at one hour with nicotinamide results in partial preservation of NAD in the islets. This corresponds well with the extent of protection against streptozotocin-induced diabetes provided by nicotinamide in relation to the time of its administration. Thus, the effect of nicotinamide in protecting against streptozotocin diabetogenicity appears to be related to its ability to antagonize the NAD-depleting effect of streptozotocin. The lack of protection by nicotinic acid against streptozotocin diabetes[3,5,6,8] may be related to its inability to prevent depletion of NAD in the islet cells, as shown in the present study. Why nicotinic acid, a precursor of NAD biosynthesis,[9,10] failed to maintain the NAD content of the pancreatic islets of the streptozotocin-treated animals remains unknown. It has been postulated that the beta cells may lack a NAD-biosynthetic pathway capable of using nicotinic acid as a precursor.[5,6] However, the nicotinic acid pathway is present in liver tissue[9,10] and nicotinic acid still is incapable of preventing streptozotocin-induced NAD depletion in that organ, as does nicotinamide.[3]

A slight decrease in the islet cell NADH following streptozotocin administration was observed, but was significant only at five hours post-treatment. In mouse liver, both NAD and NADH are markedly decreased in response to streptozotocin.[4] In the present study, however, the changes in the NADH concentration in pancreatic islets were not as striking as those of NAD.

The results suggest that the beta-cell deterioration in streptozotocin-induced diabetes may be causally related to depletion of cellular NAD. The mechanism by which streptozotocin induces NAD depletion in the islet cells remains to be investigated.

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