SIGNIFICANCE OF STREPTOZOTOCIN INDUCED NICOTINAMIDE-ADENINE-DINUCLEOTIDE (NAD) DEGRADATION IN MOUSE PANCREATIC ISLETS

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1. Introduction

Streptozotocin (*N*-methyl-nitrosocarbamyl-glucosamine) [1] an antitumor agent has been shown to induce diabetes in various animals by destroying the β-cells of pancreatic islets [2,3]. In the same animals Streptozotocin lowers liver nicotinamide-adenine-dinucleotide (NAD) content [4]. Both the diabetogenic effect and the depression of liver NAD content can be abolished by nicotinamide [5] which is known to be a precursor for NAD biosynthesis [6]. It has been suggested that Streptozotocin might also interfere with the NAD metabolism of the β-cell, and that its diabetogenic effect may be related to an inhibition of NAD biosynthesis in these cells [7].

In the present study it is demonstrated that Streptozotocin induces NAD degradation in mouse pancreatic islets, and that the depression of islet NAD content can be prevented by substances which inhibit NAD degradation by inhibition of NAD-glycohydrolase (EC 3.3.3.5).

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2. Material and methods

2.1. *Animals and incubation of islets*

Islets were isolated by collagenase digestion [8] from the pancreata of 3–4 weeks old male Swiss albino mice which were fed ad libitum. Batches of 100 islets were incubated in 1 ml of a medium containing Krebs–Ringer-bicarbonate buffer, pH 7.4, and inactivated mouse serum (4:1, v/v) in an atmosphere of 95% O_2:5% CO_2 with 250 μmole L-glutamine and 2 mg/ml D-glucose. When incorporation of labelled nicotinic acid into NAD was studied to each incubation tube 10 μCi [7-^{14}C]nicotinic acid (59 μCi/mmole, The Radiochemical Centre Amersham, England) were added. The details of the experiments are given in the legends to the figure and the tables. At the end of the incubation period the islets were washed twice in Krebs–Ringer buffer and after addition of 1 ml 0.5 N cold perchloric acid disrupted by sonification. The extract was centrifuged, 10 min × 10 000 *g*, and the supernatant used for NAD determinations.

2.2. *Determination of nicotinamide-adenine-dinucleotide*

For determination of islet NAD content a colorimetric method was used [9]. NADH produced by enzymic cycling with alcohol dehydrogenase reduces thiazolyl blue through the intermediation of phenazine methosulfate to the corresponding purple-colored formazan. The rate of reduction of thiazolyl blue is proportional to the concentration of

coenzyme. The perchloric acid extract of the islets were brought to a pH of 5.0 with 3.0 N KOH and the $KClO_4$ formed separated by centrifugation. The reaction mixture contained: 0.05 ml of a solution of 5 mg MTT/ml (thiazolyl blue, Serva, Heidelberg), 0.8 ml of a solution of 1 mg PMS/ml (phenazine methosulfate, Serva, Heidelberg), 0.1 ml of a solution of 1 mg ADH/ml (alcohol dehydrogenase from yeast, EC 1.1.1.1., specific activity 200 U/mg, Boehringer, Mannheim) and 1.95 ml of glycyl–glycine buffer, pH 7.4, that contained 0.1 M nicotinamide and 0.5 M ethanol. The mixture was warmed to 37° for 10 min and the reaction started by addition of 0.1 ml of the sample. The rate of increase in the absorbance was read after 30 and 60 min incubation at 37° against a blank at 560 nm in a Zeiss spectrophotometer PM Q II. The change in the absorbance was proportional to the coenzyme concentration in the samples which was read from a standard curve obtained with pure NAD (β-NAD, grade I, Boehringer, Mannheim) under the same conditions.

Streptozotocin (Lot Nr. 9164-UDV–136 U-9889) was a kind gift by the Upjohn Co., Heppenheim, Germany, 5-methyl-nicotinamide was kindly supplied by the Eli Lilly Co., Indianapolis, USA.

2.3. *Determination of [7-^{14}C] nicotinic acid incorporation in the NAD-molecule*

The NAD was precipitated from the $HClO_4$ extract of the islets after addition of 500 μg NAD (Boehringer, Mannheim) to each sample as carrier with a 7-fold volume of −18° acetone. For quantitative precipitation of NAD, and other dinucleotides, the samples were allowed to stand at −18° for 12 hr. After 20 min centrifugation at 40 000 *g* in a refrigerated centrifuge the sediment was washed twice with acetone and ether, respectively, and finally dissolved in 0.1 ml H_2O. Aliquots of the samples were then spotted on 20 × 20 cm thin-layer plates coated with a 0.5 mm layer of cellulose (AB, Merck, Darmstadt, Germany). Separation of nicotinic acid (NaAD)-and nicotinamide (NAD)-dinucleotides was done by electrophoresis in 0.1 M phosphate buffer, pH 6.5, with 1000 V, at ± 0°. After 2 hr a good separation of the dinucleotides had occurred which was controlled by a simultaneous run of [7-^{14}C]nicotinic labelled NaAD and NAD (own preparation). The NAD-spots were then visualized under an UV-lamp and the spots scraped off into counting vials. The radioactivity was measured in 8 ml of toluene scintillation fluid in a Packard Model 3320 liquid scintillation spectrometer. The recovery of labelled NAD in model experiments was more than 80%. For determination of the specific activity of NAD the NAD content of an aliquot was related to the counts per min found in an equal volume of the $HClO_4$ extract of the islets.

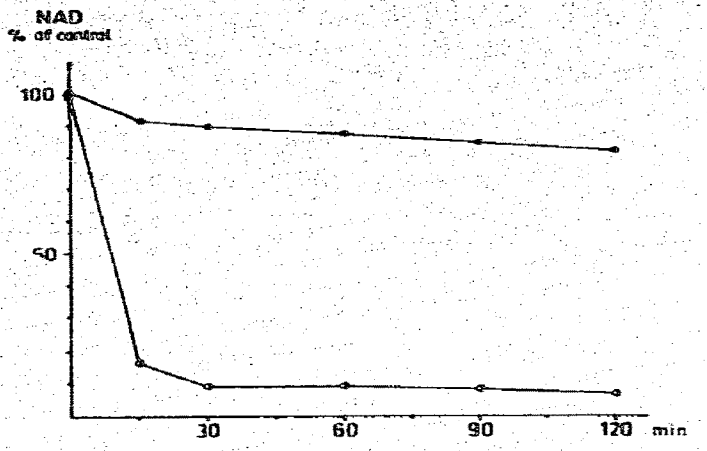

Fig. 1. Time-course of NAD-degradation in mouse islets incubated with Streptozotocin (200 μg/ml) ○—○—○ and with Streptozotocin and 5-methyl-nicotinamide (500 μg/ml) ●—●—●. Each point represents the mean value of two different experiments. In each experiment, both the control (50) and the treated islets (50) were obtained from the same collagenase-digested pancreases. For details see Methods.

Results

The assay employed for the measurement of islet NAD content is sensitive for NAD concentrations as low as 10^{-12} M. Mouse isolated islets were found to have a NAD content of 86 ± 34 pmole per 100 islets. In a few experiments the wet weight of 100 islets as used in our experiments was 109 ± 21 μg. From this it is concluded that the NAD content of mouse islets is about 800 nmole/g wet weight. Control experiments showed that the NAD concentration of isolated islets were constant and linearly related to the number of islets for at least 2 hr under the conditions used.

Incubations of islets with Streptozotocin resulted in a strong decrease of their NAD content amounting

Table 1
NAD-content of mouse islets incubated for 2 hr with Streptozotocin (Strepto.) and the preventing effect of 5-methyl-nicotinamide (5-Me-NAm) and nicotinamide (NAm).

Test	NAD (pmole/100 islets)	% of control
Control	86 ± 34	
+ Strepto. (200 μg/ml)	12 ± 5	14
+ Strepto. + 5-Me-NAm (500 μg/ml)	70 ± 19	82
+ 5-Me-NAm	96 ± 31	113
+ Strepto. + NAm (500 μg/ml)	80 ± 27	91
+ NAm	94 ± 41	109

Each value represents the average ± SEM of 6 different experiments. For details see text.

Table 2
Incorporation of [7-^{14}C]nicotinic acid in the NAD of mouse islets.

Test	cpm / 100 islets × 120 min	cpm / pmole NAD
Control	2952 ± 412	34
+ Strepto. (200 μg/ml)	309 ± 53	26
+ Strepto. + 5-Me-NAm (500 μg/ml)	2180 ± 392	31
+ 5-Me-NAm	3173 ± 456	33
+ Strepto. + NAM (500 μg/ml)	2220 ± 294	28
+ NAM	2310 ± 421	24

The effect of Streptozotocin alone and together with 5-methyl-nicotinamide and nicotinamide on the specific activity of NAD. Each value represents the average ± SEM of 6 different experiments.

to about 10% of the concentrations measured in the controls. This decrease occurred within 15 min (fig. 1). The NAD concentration then remains nearly unchanged up to 2 hr. Addition of nicotinamide and its derivative, 5-methyl-nicotinamide, which is known to inhibit the NAD-glycohydrolase (EC 3.2.2.5.) [10] totally abolishes the Streptozotocin induced NAD depression. Addition of nicotinamide or 5-methyl-nicotinamide alone leads only to a slight increase of the NAD content (table 1).

To elucidate further the mechanism of Streptozotocin induced NAD-depression incorporation of [7-^{14}C]nicotinic acid, the main precursor of NAD-biosynthesis [11], into the NAD of isolated islets was studied. Table 2 shows that Streptozotocin reduces the total amount of counts found in the NAD-molecule down to 10% of the control values. However, specific activity of NAD in the Streptozotocin experiments was not significantly different from the control ones. Addition of nicotinamide resulted in a slight decrease of the specific activity of NAD, while addition of 5-methyl-nicotinamide showed no significant effect.

4. Discussion

It has been suggested that the diabetogenic effect of Streptozotocin might be related to an effect on the NAD-metabolism of the β-cells similarly as it acts on liver NAD-metabolism [12]. The present study demonstrated that Streptozotocin induces a rapid NAD degradation in isolated mouse pancreatic islets and that this increased NAD degradation results in a decrease of islet NAD content. It seems reasonable to assume that NAD concentration we have measured is characteristic of β-cells, though this cell type accounts for only 80% of mouse islet cells [13].

That increased NAD degradation rather than inhibition of NAD biosynthesis is the mechanism by which Streptozotocin reduces islet NAD content was shown by the experiments where 5-methyl-nicotinamide was used. This derivative of nicotinamide is known to inhibit the NAD-glycohydrolase preventing in this way NAD degradation [10]. It does not act as a precursor for NAD biosynthesis like nicotinamide which has both effects [6] and therefore does not contribute to clear up the mechanism of Streptozotocin induced NAD degradation.

No evidence for an inhibition of NAD biosynthesis by Streptozotocin was found further in experiments in which the incorporation of [7-^{14}C]nicotinic acid into NAD was studied. Though there are no detailed examinations for the pathway of NAD biosynthesis in pancreatic islets, pilot experiments show that nicotinic acid rather than nicotinamide or chinolinic

acid which are known to act in other tissues as well [14] is the most important precursor for NAD biosynthesis in mouse pancreatic islets. If NAD biosynthesis were inhibited a strong decrease of the specific activity of NAD should occur [15]. However, in our experiments Streptozotocin did not alter significantly the incorporation of labelled nicotinic acid into the NAD molecule which is demonstrated by the unchanged specific activity of NAD in the experiments.

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