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PROTECTION BY PICOLINAMIDE, A NOVEL INHIBITOR OF POLY(ADP-RIBOSE) SYNTHETASE, AGAINST BOTH STREPTOZOTOCIN-INDUCED DEPRESSION OF PROINSULIN SYNTHESIS AND REDUCTION OF NAD CONTENT IN PANCREATIC ISLETS*

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SUMMARY

Picolinamide, 2-pyridinecarboxylic acid amide, was found to be a strong inhibitor of poly(ADP-ribose) synthetase of nuclei from rat pancreatic islet cells. Another experiment using isolated pancreatic islets of rats showed that picolinamide protects against streptozotocin-induced depression of proinsulin synthesis as well as against streptozotocin-induced reduction of NAD content. The protection by picolinamide against the NAD depression was considered to be due to the blockage of an increased degradation of NAD mediated by a streptozotocin-induced increase in poly(ADP-ribose) synthetase activity. A possible mechanism of streptozotocin diabetes and its prevention is discussed.

Streptozotocin, 2-deoxy-2-(3-methyl-3-nitrosoureido)-D-glucopyranose, shows a strong diabetogenic action in rat (1), mouse(2), dog (1), monkey (3) and man (4). It is known that streptozotocin inhibits proinsulin synthesis in pancreatic islets and depresses islet NAD content (5,6). As nicotinamide prevents these effects of streptozotocin (7-9), it has been so far considered that streptozotocin impairs NAD synthesis in islets to cause diabetes (10,11). However, Hinz et al. suggested that the streptozotocin-induced depression of mouse islet NAD content may be ascribed to increased NAD degradation (12).

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More recently, Doi (13) and Kazumi et al. (14) reported that picolinamide, an isomer of nicotinamide, also prevented streptozotocin diabetes in rats. Their observation also suggests that the mechanism of streptozotocin diabetes and its prevention should be sought in the degradation process of NAD, because picolinamide is structurally incapable of acting as a precursor in NAD synthesis via any biosynthetic routes (15-17).

Additionally, it has been found that nitrosourea compounds including streptozotocin increase the activity of poly(ADP-ribose) synthetase, an NAD degradating enzyme, in many eukaryotic cells (18-20). Poly(ADP-ribose) synthetase catalyzes chromatin-bound polymerization of an ADP-ribose moiety of NAD (20,21), and evidence has accumulated that this enzyme exerts a main role in NAD degradation in mammalian cells (20-23).

The present study was designed to investigate the effects of picolinamide and nicotinamide on poly(ADP-ribose) synthetase activity in nuclei from rat pancreatic islet cells and on streptozotocin-induced depression of proinsulin synthesis as well as the depression of NAD content in islets. The results raised a novel view on the biochemical basis of streptozotocin diabetes and its prevention.

MATERIALS AND METHODS

Chemicals: Nicotinamide [U-^{14}C]adenine dinucleotide (277 mCi/mmol) was purchased from the Radiochemical Center, L-[4,5-^{3}H]leucine (51.6 Ci/mmol) from New England Nuclear, streptozotocin from Upjohn Co. and picolinamide from Tokyo Kasei Kogyo Co.

Isolation of pancreatic islets: Pancreatic islets of Langerhans were isolated from male Wistar rats weighing 200-250 g as previously described (24). Rats were allowed free access to water and laboratory chow.

Isolation of islet cell nuclei and assay of poly(ADP-ribose) synthetase: About 1,000 islets were homogenized with a Kontes Micro-Tissue-Grinder in 0.25 M sucrose containing 5 mM Tris-HCl(pH 7.5), 1 mM ethylenediaminetetraacetate (EDTA), 0.5 mM ethylene glycol bis(β-aminoethyl ether)N,N'-tetraacetate (EGTA) and 3 mM $CaCl_2$ (25). The homogenate was centrifuged at 3,000 rpm for 10 min at 4°C in a Sorvall HB-4 rotor. The pellet was rehomogenized and centrifuged under the same condition. The sedimented nuclei were suspended in 100 µl of 50 mM Tris-HCl(pH 7.5) containing 30% glycerol, 1 mM EDTA and 0.5 mM EGTA (25).

The suspension (islet nuclear fraction) usually contained 14-16 μg islet nuclear DNA, which was measured by a fluorometric method (26). The assay mixture of poly(ADP-ribose) synthetase contained 100 mM Tris-HCl(pH 8.0), 10 mM $MgCl_2$, 0.2 mM NAD^+, 1 mM dithiothreitol, 5.6×10^{-2} μCi of [^{14}C]-NAD^+ and an appropriate amount of the islet nuclear fraction in a final volume of 100 μl. The enzyme activity was determined by measuring the incorporation of [^{14}C]-NAD^+ into acid-insoluble materials (25).

Proinsulin synthesis in islets: Batches of 30 islets were incubated for 60 min at 37°C in 100 μl of Krebs-Ringer bicarbonate medium supplemented by 2 mg/ml bovine serum albumin and 20 mM glucose in the presence of 10 μCi [^{3}H]-leucine (27,28). The amount of [^{3}H]-proinsulin synthesized was determined by SDS-polyacrylamide gel electrophoresis as described previously (29,30).

Determination of islet NAD content: NAD content of incubated islets was measured by the procedure of Hinz et al. (12). The value was corrected for overall recovery by the addition of [^{14}C]-NAD^+.

RESULTS

It has been reported that nicotinamide is a strong inhibitor of poly(ADP-ribose) synthetase in various eukaryotic cells (20,21). In the present study, picolinamide was found to inhibit islet nuclear poly(ADP-ribose) synthetase in a dose-dependent manner (Fig. 1). The concentration for 50% inhibition was about 0.1 mM. The enzyme activity was nearly completely inhibited by 2 mM

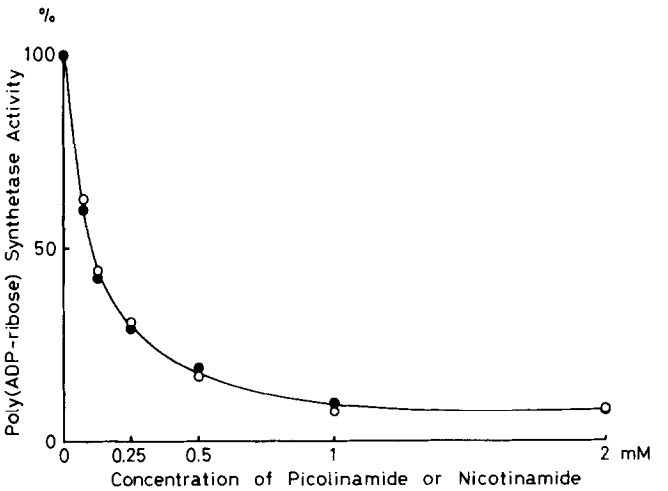

Fig. 1. Inhibition of poly(ADP-ribose) synthetase of islet cell nuclei by picolinamide or nicotinamide. Various amounts of picolinamide (●—●) or nicotinamide (O—O) were added in the assay mixture described in MATERIALS AND METHODS. The reaction was carried out at 25°C for 10 min and stopped by the addition of 0.1 ml of cold 20% trichloroacetic acid (TCA). Acid-insoluble materials were collected on Millipore filters, washed extensively with cold 10% TCA and counted in a toluene-based scintillation solution. All activities were related to that of an equivalent quantity of islet nuclear fraction without inhibitor (12.5 pmoles poly(ADP-ribose) synthesized/10 min/μg islet nuclear DNA).

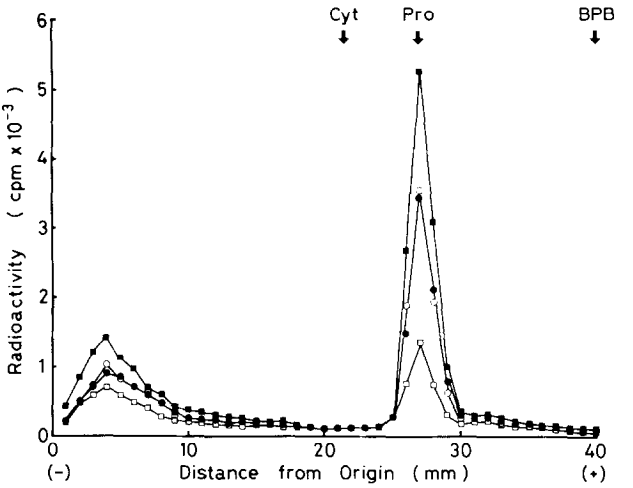

Fig. 2. SDS-Polyacrylamide gel electrophoresis of [^{3}H]-labeled products in pancreatic islets. Islets were incubated as described in MATERIALS AND METHODS: ■—■, control without streptozotocin; □—□, with 0.5 mg/ml streptozotocin; ●—●, with 0.5 mg/ml streptozotocin and 2 mM picolinamide; ○—○, with 0.5 mg/ml streptozotocin and 2 mM nicotinamide. Incubated islets were washed with cold Hanks solution, sonicated in 200 µl of 60 mM Tris-phosphoric acid(pH 6.8) containing 1% SDS, 5% 2-mercaptoethanol and 10% glycerol, and heated at 100°C for 2 min. An 80-µl aliquot of the heated sample was submitted to SDS-polyacrylamide(15%) gel electrophoresis. The amount of proinsulin synthesized was calculated by summing the radioactivity incorporated in the proinsulin peak (28-30). Arrows indicate relative migration of: Cyt, horse heart cytochrome c (MW 13,400); Pro, rat proinsulin (MW 9,000); and BPB, bromophenol blue, run on parallel gels.

picolinamide. Nicotinamide also inhibited islet nuclear poly-(ADP-ribose) synthetase. The inhibition curve of poly(ADP-ribose) synthetase activity by picolinamide was almost identical to that by nicotinamide, indicating that picolinamide is just as strong an inhibitor of the enzyme as nicotinamide.

As proinsulin synthetic ability of islets seems to be a crucial marker for evaluation of diabetogenicity, we examined the effects of streptozotocin, picolinamide and nicotinamide on proinsulin synthesis in isolated islets (Fig. 2). Islets were incubated in the presence of 20 mM glucose throughout the experiment, because the proinsulin synthesis is greatly stimulated at that glucose concentration (28,30). Proinsulin synthesized in islets comprised more than half of the total protein synthesized and gave a major peak on SDS-polyacrylamide gel electrophoresis.

Table 1

Effects of picolinamide or nicotinamide on streptozotocin-induced reduction of islet NAD content

Addition	NAD Content
	pmole/100 islets
none	252 (100)
+ 0.5 mg/ml streptozotocin	33 (13)
+ 0.5 mg/ml streptozotocin and 2 mM picolinamide	355 (141)
+ 0.5 mg/ml streptozotocin and 2 mM nicotinamide	418 (166)

Batches of 100 islets were incubated under the same condition as for determination of proinsulin synthesis except for omitting [^{3}H]-leucine. After incubation, islets were washed with Hanks solution and disrupted by sonication in 1 ml cold 0.5 N perchloric acid. The acid-soluble extract of islets was brought to pH 5.0 with KOH and used for NAD assay. NAD content was assayed as previously described (12). [^{14}C]-NAD$^+$ was added to each sample before disruption of islets and its recovery in the final preparation was referred to for correction of the NAD value for overall recovery. The numbers in parentheses give the percentage of NAD content without streptozotocin.

Proinsulin synthesis in islets incubated with 0.5 mg/ml streptozotocin decreased to about 25% of the control without streptozotocin. As previously reported (5,9), addition of 2 mM nicotinamide reversed the decrease to about 75% of the control. Addition of 2 mM picolinamide was found to reverse the decrease to almost the same extent as that with nicotinamide. The inhibitory effect of 0.1 mg/ml streptozotocin on proinsulin synthesis (70% of the control) was completely abolished by the addition of either 2 mM picolinamide or 2 mM nicotinamide (data not shown).

Islet NAD content was markedly reduced when incubated with 0.5 mg/ml streptozotocin (Table 1). On the other hand, islets incubated under coexistence of streptozotocin and picolinamide (or nicotinamide) showed a slight increase in intracellular NAD content in comparison with islets incubated in the absence of streptozotocin.

DISCUSSION

In the present work, the presence of poly(ADP-ribose) synthetase has been clearly demonstrated in rat pancreatic islet nuclei and picolinamide was found to be a potent inhibitor of the enzyme. Further experiments with isolated islets showed that picolinamide can protect proinsulin synthesis as well as the intracellular NAD level against streptozotocin-induced depression.

That increased NAD degradation rather than inhibition of NAD biosynthesis is the mechanism by which streptozotocin reduces islet NAD content was shown through the experiment with picolinamide, since picolinamide is incapable of acting as a precursor in NAD synthesis. However, as the addition of nicotinamide led to a somewhat larger increase in islet NAD content than that with picolinamide (Table 1), the possibility that nicotinamide can serve as a precursor in NAD synthesis in islets was not excluded.

A temporal correlation between an increase in poly(ADP-ribose) synthetase activity and a decrease in intracellular NAD content was found in mouse leukemia cells treated with N-methyl-N-nitrosourea (31). More recently, Juarez-Salinas et al. showed that an equimolar increase in the intracellular level of poly-(ADP-ribose) coupled with an NAD reduction actually occurs in vivo (32).

We have already found that incubation of islets with streptozotocin leads to a significant increase (2-3 fold) in islet nuclear poly(ADP-ribose) synthetase activity (33).

Therefore, it is reasonable to assume that streptozotocin causes an increased flux from NAD through poly(ADP-ribose) by increasing poly(ADP-ribose) synthetase activity to depress islet NAD content and inhibit islet cell functions including proinsulin synthesis. Inhibitors of poly(ADP-ribose) synthetase such as

picolinamide and nicotinamide may prevent the diabetogenic action of streptozotocin by maintaining the intracellular NAD level. This concept may answer the controversy about why nicotinic acid cannot prevent streptozotocin diabetes (7). Nicotinic acid acts as a precursor for NAD synthesis but is proven not to inhibit poly(ADP-ribose) synthetase (34).

Poly ADP-ribosylation has been closely correlated with DNA synthesis (35), DNA repair (19), cell differentiation (36) and carcinogenesis (37). We have presented here the evidence that poly ADP-ribosylation may also be involved in the etiology of diabetes.

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