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COMMENTARY

Potential Role of Environmental Genotoxic Agents in Diabetes Mellitus and Neurodegenerative Diseases

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ABSTRACT. Epidemiological data suggest that environmental genotoxins are risk factors for some forms of diabetes mellitus and neurodegenerative diseases. The present commentary focuses on mechanisms involved in genotoxin-induced pancreatic β-cell and neuronal damage. These two cell types seem to share a similar vulnerability to different forms of DNA damage, and the long-term consequences of repeated genotoxic insults to post-mitotic neurons or slowly proliferating β-cells remain to be clarified. One intriguing possibility is that genotoxins could act as "slow" toxins in these cells, triggering a cascade of cellular events, which culminates in progressive cell dysfunction and loss. Indeed, exposure to mutagenic nitroso agents such as streptozotocin and cycasin induces long-lasting damage to both β-cells and neurons. These data on cycasin, a toxin obtained from the cycad plant (*Cycas* spp.), are of special interest, since this agent may be implicated in both amyotrophic lateral sclerosis/Parkinson dementia complex and diabetes mellitus in the western Pacific area. Future studies are required to sort out the interactions between different genotoxic agents, viral infections, and cellular repair mechanisms on cellular survival and function. Moreover, further epidemiological studies are needed to clarify the role of *N*-nitrosoureas in diabetes mellitus and neurodegenerative diseases in populations with different genetic backgrounds. Answers to these questions may provide useful information on the pathogenesis of these devastating diseases, and open the possibility for their primary prevention. BIOCHEM PHARMACOL 51;12:1585–1591, 1996.

KEY WORDS. diabetes mellitus; neurodegenerative diseases; pancreatic islets; DNA damage; DNA repair; *N*-nitrosamines; cycad; streptozotocin; methylazoxymethanol; cycasin

This commentary focuses on some of the mechanisms that may be involved in genotoxin-induced pancreatic β-cell and neuronal damage. These two cell types have several features in common. Thus, each of them has a low capacity for cell proliferation in the adult state [1, 2], and each depends on a high rate of oxidative metabolism. Furthermore, pancreatic β-cells and neurons express receptors for nerve growth factor [3], are electrically excitable upon stimulation [4, 5], contain high levels of the enzyme glutamic acid decarboxylase, and synthesize γ-aminobutyric acid [6]. Morphologically they are also able to display neurite-like processes when dissociated into single cells [7]. It is also noteworthy that both in inherited diseases affecting mitochondrial DNA [8, 9] and in ALS/PDC‖ [10–12], neurological dysfunction and hyperglycemia are common outcomes. This suggests that β-cells and neurons share a similar vulnerability to different forms of genetic damage, mutations, and/or deletions. In this respect, a key issue may be the low proliferative capacity of these cells. Most studies have focused on the effects of environmental genotoxins on dividing cells and their consequent carcinogenic properties [13]. However, the long-term consequences of repeated genotoxic insults to post-mitotic neurons or slowly proliferating β-cells are unknown. One intriguing possibility is that genotoxins could act as "slow" toxins in these cells, triggering a cascade of cellular events, which culminates in progressive cell loss [12, 14].

EPIDEMIOLOGICAL DATA ON ENVIRONMENTAL GENOTOXINS, DIABETES MELLITUS, AND NEURODEGENERATIVE DISEASES

IDDM is an autoimmune disease, involving an attack by T cells, macrophages, and B-cell-derived antibodies against pancreatic β-cells [15]. Environmental factors may play a major role in IDDM induction [16]. Epidemiological studies have shown age, seasonal, sex, and geographical variability of disease occurrence. There is also an increasing IDDM risk with time both within stable populations and among migrant groups. These observations, and the low concor-

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‖ *Abbreviations:* ALS/PDC, amyotrophic lateral sclerosis/Parkinson-dementia complex; IDDM, insulin-dependent diabetes mellitus; MAM, methylazoxymethanol; MMS, methyl methanesulfonate; NIDDM, non-insulin-dependent diabetes mellitus; NMU, nitroso-*N*-methylurea; NO, nitric oxide; O^6-mdGuo, O^6-methyldeoxyguanosine; PARP, poly(ADP-ribose) polymerase; and SZ, streptozotocin.

dance rate of IDDM among monozygotic twins (30–50%), suggest that genetic risk factors may be necessary but are not sufficient for diabetes to occur [17]. Among the environmental agents that may contribute to diabetes, special attention has been devoted to infectious agents (mostly viruses) [18], diet, and environmental toxins. There are descriptive epidemiological data suggesting that *N*-nitroso compounds from smoke-cured mutton may contribute to an increased prevalence of IDDM in Iceland [19, 20]. Moreover, a case-control study in Sweden has shown a dose-response relationship between the risk for IDDM and intake of food containing nitrosamines [21], and an ecological study from the U.S.A. showed correlation between the content of nitrate in drinking water and the incidence of IDDM [22]. Interestingly, new data suggest that ingestion of mutagenic nitroso compounds from betel nuts [23] or azoxyglycosides (i.e. cycasin: MAM β-D-glucose) from the cycad plant *Cycas circinalis*, L. [24] may be related to increased prevalence of NIDDM in the Pacific area.

Regarding experimental data, SZ, an *N*-methyl-*N*-nitroso-urea derivative of 2-deoxyglucose, is a well-known diabetogenic agent in rodents [25, 26]. Besides being selectively toxic to β-cells, repeated subdiabetogenic injections of SZ can induce an immune response against these cells in certain strains of mice [25, 27, 28]. Subdiabetogenic doses of SZ to rodents also induce a reduction in brain energy metabolism [29, 30], alter cholinergic neurotransmission [31, 32], and impair memory [33, 34]. These features are similar to those found in certain chronic neurodegenerative disorders (e.g. Alzheimer's disease) [35].

In neurodegenerative diseases, there is increasing evidence that environmental agents may contribute to the demise of neurons in ALS, Parkinson's disease, and Alzheimer's disease. The finding that initiated the intensive search for environmental toxins in these disorders was the discovery in the late 1970s that the drug contaminant 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induces in intravenous drug users neurological features resembling idiopathic Parkinson's disease [36], a finding that could be reproduced in various animal models. Moreover, for the past several decades, investigators have been trying to identify the etiological trigger of a prototypical neurological disorder found in the Chamorro people in the western Pacific, with features of ALS (Lou Gehrig's disease), Parkinsonism (P), and an Alzheimer-like dementia (D) (ALS/PDC) [14, 37]. Since the disease incidence is declining or has disappeared among young people in all three affected western Pacific areas [37–40], and heritable and infectious agents have been virtually ruled out [41, 42], the search for the etiology of this disorder has focused on non-transmittable environmental factors that are vanishing as the susceptible populations adapt to "modern" ways. Two major hypotheses for the etiology of this disorder have been proposed: (i) a deficiency in nutritional intake of calcium in conjunction with an increased absorption of aluminum and other metals [37, 38], and (ii) exposure to the toxic cycad plant used for food and medicine [42, 43]. The inability to detect abnormalities in calcium metabolism or heavy metal absorption in Guam ALS/PDC subjects [44] and the failure of animals chronically treated with aluminum to develop clinical and neuropathological signs characteristic of ALS/PDC [37] suggest that aluminum is unlikely to be the principal agent causing the disease, but it may still be a contributing factor. In contrast, an epidemiological study has shown that preference for traditional Chamorro food was the only one of 23 tested variables significantly associated with an increased risk for Guam ALS/PDC [45]. This is consistent with the hypothesis that the seed of the neurotoxic cycad plant (*C. circinalis*, L.), a traditional but declining source of food and medicine for the Chamorro people, plays a role in the etiology of western Pacific ALS/PDC. The cycad hypothesis has been strengthened by recent studies that demonstrate a direct correlation between: (i) the presence of ALS/PDC in populations exposed to cycad toxins [39, 40, 46]; and (ii) the absence of ALS/PDC in groups exposed to cycad lacking detectable concentrations of its constituent toxin [43, 47]. It is noteworthy that cycasin, the predominant neurotoxin in cycad, and the diabetogenic agent SZ (see above) are chemically similar in that both contain a glucoside and a potent genotoxin.

MECHANISMS OF LONG-LASTING β-CELL AND NEURONAL DAMAGE BY GENOTOXINS

Non-lethal doses of SZ induce a long-term impairment in the capacity of rodent β-cells *in vitro* to express insulin mRNA, and to produce and release insulin, while other basic cellular functions are much less affected [48–50]. Even when these SZ-treated cells are transplanted into normoglycemic animals, and maintained in the host animal for several weeks, they fail to regain normal insulin release [51, 52]. These effects of SZ seem rather unique, since pancreatic islets exposed to alloxan [53], interleukin-1 [54], and heat shock [55] are able to regain their function after several days in tissue culture. The SZ-induced dysfunction is probably related to damage to the mitochondrial genome [52, 56–59], impairment of FAD-linked glycerophosphate dehydrogenase and NAD-dependent 2-ketoglutarate dehydrogenase [60], defective substrate catabolism at the mitochondrial level [50], and decreased ATP generation [49]. Since ATP production is a crucial component in the signal transduction of glucose-induced insulin release [61], a decrease in cellular ATP may contribute to the observed β-cell dysfunction [48, 49]. However, genomic mitochondrial damage is unlikely to be the sole mechanism, since two other methylating agents, MMS and NMU, also induce long-lasting damage to mitochondrial DNA without causing prolonged β-cell dysfunction [58]. An important difference between SZ and MMS or NMU is the presence of a glucose moiety in SZ, suggesting the intriguing possibility that this glucose moiety will "target" the *N*-nitrosourea to intracellular sites of β-cells involved in the signal transduction for glucose-induced insulin release [26, 62]. Moreover,

the selective cytotoxic effects of SZ on neuroendocrine cells overexpressing GLUT-2, which is the main glucose transporter of mature rodent β-cells, suggest that glucose transport is also essential for the deleterious effects of SZ on β-cells [63].

The decomposition of SZ and NMU releases highly reactive carbonium ions, causing widespread alkylation of DNA bases [64, 65]. DNA alkylation can be followed by excision DNA repair, inducing DNA strand breaks and activation of the enzyme PARP. PARP consumes NAD^+, and NAD^+ depletion has been suggested to play a pivotal role in β-cell death following acute exposure to SZ [66]. However, pancreatic islets exposed to SZ regain a normal cellular NAD^+ content, but not a normal insulin release, after 6 days in culture, arguing against NAD^+ depletion as the cause for prolonged β-cell dysfunction [58]. An alternative hypothesis is that acute exposure to SZ, and maybe repeated exposure to other environmental nitrosoureas, may lead to lasting DNA alkylation. In this context, SZ may act like the radiosensitizer bromodeoxyuridine, which early in life has been shown to induce signs of premature aging (i.e. early loss of hair, body weight, abnormal teeth) in rats and influence the carcinogenic (and presumably DNA damaging) properties of nitrosoureas given later in life [67]. Moreover, bromodeoxyuridine sensitizes cultured mouse brain cortical tissue to MAM-induced DNA damage [68]. One important site of DNA alkylation following islet exposure to SZ or MAM (see below) is the exocyclic oxygen atom of guanine, forming O^6-mdGuo [26, 65]. O^6-mdGuo can persist for long periods, and it is correlated with mutagenesis and carcinogenesis in actively proliferating tissue [69]. However, it is unclear how these DNA adducts affect cells with low proliferative capacity, such as β-cells and neurons. O^6-mdGuo is repaired by alkylguanine alkytransferase, a DNA repair enzyme with low activity in pancreatic β-cells [70] and post-mitotic neurons [68], and DNA adducts have been shown to be incompletely removed from β-cell DNA 24 hr after genotoxin exposure [70]. The ability of MAM to reduce DNA repair significantly (e.g. apurinic/apyrimidinic endonuclease, APE) in terminally differentiated human neuroblastoma cells [68, 71] and mature rodent neuronal tissue [72] suggests that the long-term impairment of neuronal or β-cell function by cycad genotoxins or SZ may also be mediated by their ability to both damage and alter DNA repair. It is therefore conceivable that some types of alkylated nucleic acids may lead to an irreversible epigenic change that culminates in defective or modified gene expression. This could lead to either cell degeneration or expression of modified proteins and recognition of these cells as "non-self" by the immune system, with consequent cell destruction. In favor of the second possibility are the observations that repeated subdiabetogenic injections of SZ induce an immune assault against β-cells in some strains of mice [25, 28]. Note that abnormalities in the immune system are not considered a primary event in triggering neurodegenerative disease.

Recently it has been shown that the cycad-derived genotoxin cycasin, or the aglucone form MAM, is toxic to cultured rodent and human islets of Langerhans *in vitro* [24]. These findings are of interest, because chronic ingestion of the cycad seed may be related to the high prevalence of ALS/PDC (see above) and NIDDM in the western Pacific. Cycasin has some similarities to SZ, containing both a glucoside and an alkylating moiety. MAM, like SZ, severely damages β-cell DNA, which may contribute to cell dysfunction [24].

Rodent cell culture medium incubated with MAM only accumulates nitrite [24] (nitrite is a stable product of NO reaction with molecular oxygen), suggesting that the cycad toxin, like SZ [73, 74], probably releases NO [24]. NO is also formed intracellularly in rat hepatocytes and islet cell cultures treated with SZ [75], and rodent islets are particularly susceptible to the noxious effects of high NO concentrations [76]. The observations that the NO scavengers hemin and Fe^{2+}/diethylthiocarbamate protect mouse and rat islets from MAM [24] and SZ-induced cytotoxicity [75], respectively, suggest that intracellular NO may play an important role for the cytotoxic effects of these genotoxins in β-cells and neurons. Furthermore, NO-induced cytotoxicity in SZ-treated hepatocytes and islet cells is associated with production of DNA strand breaks [75]. NO reacts with secondary and tertiary amines, forming nitrosamines that may be metabolized to reactive species and methylate guanine to form the DNA adduct O^6-mdGuo [77]. Thus, NO release may be a novel mechanism by which some environmental nitrosoureas, or the cycad genotoxin cycasin/MAM, contribute to β-cell and neuronal damage. Interestingly, NO may also be an important mediator of immune-induced β-cell destruction in IDDM [78, 79]. This radical may therefore act at two different stages in the evolution of IDDM: (i) participating in the initial environmental insult against the β-cells; and (ii) as a mediator of cell damage in the subsequent immune assault against the β-cells.

Most of the observations discussed above were made in rodent models. Recent data, however, indicate that human pancreatic islets are less susceptible than rat or mouse islets to the deleterious effects of SZ, NO donors, alloxan [80], cycasin [24], or combinations of cytokines [81]. For example, high concentrations of SZ (12 mM) are needed to acutely inhibit human islet insulin release, while 1–3 mM SZ severely decreases rodent islet function. These findings are in line with observations that other human cell types present an increased DNA repair capacity as compared with rodent islets [82]. Furthermore, human pancreatic islets express higher amounts of the heat shock protein 70 (hsp70) and of the antioxidant enzymes superoxide dismutase and catalase than rodent islets [83]. Increased expression of hsp70 protects rodent islets against the toxic effects of interleukin-1 [84] and SZ [85]. Although it is unknown which mechanism(s) is responsible for the protective effects of hsp70, these findings indicate that the potential deleterious effects of environmental genotoxins may depend upon both

toxin exposure and repair mechanisms activated by the injured cell [86]. In this respect, it is noteworthy that there are large interindividual variations among humans in carcinogen metabolism, DNA adduct formation, and DNA repair [87].

As discussed above, there are limited circumstantial data suggesting that consumption of alimentary nitrosamides is related to increased incidence of either IDDM or NIDDM. For IDDM, this correlation was observed in Western countries, such as Sweden [21], Iceland [19], and the U.S.A. [22], whereas a possible effect on NIDDM was suggested for Asiatic populations [23] and habitants of some western Pacific islands [24]. Available epidemiological data suggest that IDDM incidence in different countries is higher among the Caucasian population than among Mongoloids and Blacks, probably reflecting the importance of different genetic susceptibility between populations [88]. As mentioned above, repeated subdiabetogenic doses of SZ induce insulitis and severe hyperglycemia in certain strains of mice. However, most mouse strains will not present the immune component of this experimental model of IDDM, suggesting that SZ-triggered immune-mediated diabetes will occur only in the presence of a particular genetic background [25, 27, 28]. If these observations can be extrapolated to humans, it appears likely that repeated exposures to alkylating agents may trigger an autoimmune assault against β-cells only in genetically susceptible populations. In populations not susceptible to such autoimmune response, repeated β-cell injury would fail to induce autoimmune IDDM, but instead might trigger a progressive loss of β-cell function, eventually evolving into a form of NIDDM.

CONCLUSIONS AND FUTURE RESEARCH DIRECTIONS

The suggestion that environmental genotoxins contribute to induction of diabetes mellitus has been a matter of interest for more than 20 years [89]. However, it has been difficult to establish a definitive relationship between environmental genotoxins and disease, probably because of the presence of several confounding factors. While most experimental studies with β-cells or neurons have utilized exposure to a single genotoxin, human exposure to these agents results from several distinct sources and over a large range of different concentrations. Moreover, individual susceptibility to environmental toxins may depend upon the different capacity for cellular defense and/or repair, the redox state of the cell, micronutrient deficiencies, and the concomitant presence of other factors, such as prior exposure to DNA-damaging agents (see discussion on bromodeoxyuridine), viral infection, or immune stimulation. In support of this hypothesis, transgenic mice expressing mB7-1 under the control of the insulin promoter (mB7-1 can provide a "costimulatory" signal for T-cell activation) are unusually sensitive to SZ-induced insulitis and diabetes [90], and combined injection of SZ and Freund's adjuvant potentiates β-cell damage [91]. SZ may induce retroviral expression in pancreatic β-cells [25], and recent data suggest that a specific nutritional deficiency, i.e. selenium deficiency, can cause changes in the viral genome, which in turn can change an avirulent strain of virus into a virulent one because of a genetic mutation [92].

In conclusion, there is sufficient epidemiological data to suggest a role for environmental genotoxins in diabetes mellitus and neurodegenerative diseases. Moreover, there is also enough cellular and molecular biological data to draw some conclusions regarding possible mechanisms involved in long-term β-cell and neuronal dysfunction. However, several questions remain to be answered. Among them, why are some genes more affected than others by DNA damage and what are the molecular mechanisms underlying the different sensitivity of various tissues, individuals, and species to alkylating agents. Detailed studies will be required to sort out the interaction between different genotoxic agents, cellular repair mechanisms, and concomitant viral infection on the outcome of cellular survival and function. More epidemiological studies are necessary to clarify the role for *N*-nitrosoureas in IDDM, NIDDM, and neurodegenerative diseases in populations with different genetic backgrounds. Eventual answers to these questions will undoubtedly increase our understanding of the pathogenesis of these devastating human diseases, and may open the possibility for their primary prevention.

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