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Nitric oxide and neuronal and pancreatic beta cell death

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Abstract

Neural cells are found in all organs of the body and play an important role in the maintenance of the internal milieu. The pancreatic beta cell is the most numerous cell types in the endocrine pancreas. It is particularly important because of its role in insulin secretion, a crucial hormone in glucose metabolism. In view of this, the significance of the survival of neural and pancreatic beta cell cannot be over emphasised. Neural and pancreatic beta cell death occurs in a variety of ways. The destruction of neural cells can be induced with (1) free radicals (H_2O_2, O_2^-, HO^-) and nitric oxide; (2) Cytokines (tumour necrosis factor, interleukin-1 beta, interferon-gamma); (3) Glutamate; (4) Amphetamine analog (Ecstasy); (5) S100 protein; (6) Ammonia; (7) Iron ions; (8) Resins, e.g. methylmethycrylate. Pancreatic beta cell can be destroyed by (1) free radicals (H_2O_2, O_2^-, HO^-) and nitric oxide; (2) Cytokines (tumour necrosis factor, interleukin-1 beta, interferon-gamma); (3) alkylating agents (streptozotocin, alloxan, *N*-methyl-nitrosourea *N*-ethyl-*N*-nitrosourea, Methylmethanesulphonate and ethylmethanesulphonate); (4) hyperglycaemia; (5) islet amyloid poplypeptide; and (6) Inositol Monophosphate dehydrogenase inhibitors. There is enough evidence that most of these agents involved in neural and pancreatic beta cell death exert their toxic effects through the nitric oxide pathway. Neuroprotective agents include vitamin B12 analogs and alpha-tocopherol, NOS inhibitors, antioxidants (e.g. glutathione, superoxide dismutase), metals like cobalt, neurotrophic receptors (Akt kinase) and growth factors. The pancreatic beta cell death induced by these toxic agents can be prevented and or delayed by nicotinamide (vitamin B3), heat shock, copper, alpha-tocopherol (vitamin E), succinic acid, dihydroxylipoic acid, fusidic acid, glucocorticoids, cyclosporin A, growth factors and gene therapy.

Keywords: Neural; Neuroprotective agents; Pancreas; Insulin; Beta cell death; Nitric oxide; Cytokines; Alkylating agents; Islet amyloid polypeptide; Heat shock; Antioxidant; Nicotinamide

1. Introduction

Nitric oxide (NO) is one of the most potent substances released from the vascular epithelium. It is a simple radical gas, signaling molecule and neurotransmitters that can heal or kill. NO is produced by the oxidation of L-arginine with the

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use of oxygen. L-citrulline is a byproduct of the reaction. The reaction is catalysed by a NADPH and Ca^{2+}/calmodulin dependent enzyme, nitric oxide synthase. In the past 10 years, NOS- and NADPH-d-positive cells have been shown in different regions of the central and peripheral nervous and other systems (Scott et al., 1987; Nakamura et al., 1988; Mizukawa et al., 1989; Schober et al., 1989; Mufson et al., 1990; Satoh, 1990; Shuttleworth et al., 1993; Adeghate et al., 1995a,b; Adeghate and Parvez, 1998). NO mediates several physiological processes in a range of cell and tissue types. NO has been implicated in the process of neural and endocrine cell death.

1.1. General actions of NO

NO which was formerly described as an endothelium-derived relaxing factor (Palmer et al., 1987) is not stored in granules and can diffuse easily from its sites of formation. This is a departure from the classical mediator concept (Nathan, 1992; Snyder, 1992). Classical mediators have more complex structures, frequently stored in granules and released specifically. NO is also soluble in water and lipid, thereby enhancing a free diffusion in the environment of the cell. NO reacts with O_2 to form a strongly oxidising molecule that is more toxic than the NO itself making it a killer molecule in the immune system when they are released from immune cells. NO has the capacity to increase the surface area of immune cells several times its original size so that they could enfold their prey before killing it with a shower of NO (Anggard, 1994). In addition to this, it has been reported that NO can disrupt mitochondrial respiration (Brorson et al., 1999). This action will deprive the cells of energy source and eventually lead to cell death.

1.2. NO and the cardiovascular system

NO is released from the vascular epithelium (Calver et al., 1992). It is formed in the conduit arteries from L-arginine in the endothelium and released after stimulation with acetylcholine. The NO released causes vasodilatation. Drugs used in pectoral angina (glyceryl trinitrate) acts to relieve angina by releasing NO in the vascular wall, which subsequently initiates the dilatation of blood vessels. This dilatation reduces resistance in arterioles and consequently reduces blood pressure. It has also been shown that there is an abnormality in the L-arginine NO system in hypertension because endothelium dependent dilation is diminished in both animals with experimental hypertension and in patients with essential hypertension (Calver et al., 1992). NO also plays an important role in the prevention of atherosclerosis by inhibiting platelet aggregation in the presence of prostacyclin (Radomski et al., 1987; Vallace et al., 1989; Kubes et al., 1991).

1.3. NO and the respiratory system

Nitric oxide has been detected in the lung and could be a physiological mediator in the respiratory system (Jorens et al., 1993). It has been suggested that it is a mediator of nerve-dependent bronchodilation (Barnes, 1993). Inhaled NO has been shown to be a cure for hypoxic pulmonary vasoconstriction (Higenbottam, 1993). High concentrations of NO can bring its own risks, ranging from increased capillary permeability to methaemoglobinaemia.

1.4. NO and the genital system

NOS have been demonstrated in the pelvic neurons innervating the corpora cavernosa and in the neuronal plexuses of the adventitial layer of the penile arteries (Burnett et al., 1992). NOS inhibitors abolished electrophysiologically produced penile erection in laboratory animals and in man (Raifer et al., 1992). The muscles of the corpora cavernosa of the penis relax in response to stimulation of NANC nerves to cause penile erection. These NANC nerves have now widely been accepted as NO. This action of NO is now used in the treatment of impotence (Viagra®). Nitroglycerin, a NO-releasing drug, has been shown to diminish the force of contractions of rat uterus myometrium strips in vitro (Diamond and Marschall, 1969). Recently, intravenous administration of glycerin has come into clinical use as a potent cervico-uterine relaxant in postpartum pa-

tients with retained placenta or a tightly contracted inverted uterus (Peng et al., 1989; DeSimone et al., 1990; Altabef et al., 1992).

1.5. NO and the central nervous system

Nitric oxide and NADPH-diaphorase has been shown in different parts of the brain. High concentrations of enzyme- and immunoreactivity for these substances have been recorded in the granular layer of the cerebellum (Snyder, 1992). NO has been suggested to have an important role in synaptic plasticity and regulates some of the neurophysiological phenomenon underlying memory and depression (Snyder, 1992; Zorumski and Igumi, 1993). NO is also involved in the coupling of local levels of neuronal activity in the brain to local blood level flow and to facilitate the release of neurotransmitters from surrounding synapses (Anggard, 1994). NO has protective roles in preventing neurons from degeneration (Snyder, 1992). On the other hand high concentrations of NO causes neuronal destruction.

2. Neural cell death

2.1. Substances causing neuronal cell death

2.1.1. Nitric oxide

NO is a neuronal messenger molecule, which interact with surrounding neural cells by diffusion between cells. NOS, which regulates NO production is activated by the influx of calcium from glutamate-activated *N*-methyl-D-asparate (NMDA) receptors (Dawson, 1995). The other way in which NO destroys neural cells is by the activation of the nuclear protein, poly (ADP-ribose) synthethase. NO may also destroy neurons by increasing the intracellular pH and or endonuclease activity (Vincent et al., 1999) (Fig. 1).

2.1.2. Cytokines

Microglial and meningeal fibroblasts can be stimulated by cytokines to produce toxic concentration of NO (Boje and Arora, 1992). These cytokines, including tumour necrosis factor (TNF), interleukin-beta (IL-β) and interferon-gamma (IF-γ) can cause apoptosis of neural cells (Downen et al., 1999). Cytokines exert their neurotoxicity via NO (Hu et al., 1997a).

2.1.3. Glutamate

Glutamate acts through NMDA receptors to increase intracellular calcium. An abnormal increase in intracellular calcium will in turn activate phospholipases, proteases and NOS and eventually lead to neuronal cell death (Stout et al., 1998; Castillo, 1999).

2.1.4. Free iron ions

Non-protein bound free iron ions have been said to be the strongest and most dangerous generators of free oxygen radicals. These oxygen radicals will in turn contribute to the destruction of neurons (Bertrand et al., 1997).

2.1.5. S100 proteins

Although S100 protein has been implicated in the development and maintenance of the nervous system, high concentrations of S100 protein can induce iNOS and hence NO. The NO released causes neuronal death (Hu et al., 1997b).

2.1.6. Mitochondrial inhibitors (e.g. 1-methy-4-phenylpyridinium)

Mitochondrial inhibitors can trigger indirect excitotoxic processes, which can lead to increase in intracellular level of calcium, protease activation and subsequent neuronal apoptosis (Leist et al., 1998).

2.1.7. Acidosis

The development of acidosis facilitates the entry of calcium into neurons. An increase in intracellular calcium will activate phospholipases, proteases and NOS and eventually lead to neuronal cell death (Castillo, 1999).

2.1.8. Liposaccharides

Microglial and meningeal fibroblasts can be stimulated by liposaccharides to produce toxic concentration of NO (Boje and Arora, 1992). Liposaccharides are released by bacterial endotoxins.

2.1.9. Amphetamine analogues [3,4-methylenedioxymethamphetamine (ecstasy)]

Ecstasy causes hallucination and psychostimulation as well as long-term neuropsychiatric disorders. Is has been shown to be cytotoxic to serotonergic neurons in rodents and monkeys. The effect in humans is less clear (Simantov and Tauber, 1997).

2.1.10. Methylmethycrylate (MMA)

MMA has been shown to cause apoptosis in human embryonic neocortical neurons (Chen et al., 1998). This neurotoxic action of MMA is mediated through free radicals. This report by Chen and others is the first report implicating resins used in dental practice in the aetiology of neuronal cell death.

2.2. Prevention of neuronal cell death

2.2.1. NOS inhibitors

Daily administration of *N*-omega-nitro-L-arginine-methyether (L-NAME) protected neurons from cell death (Ruan et al., 1995). The ability of NOS inhibitors to prevent neuronal cell death indicates that NO is involved in may pathways leading to neuronal cell death.

2.2.2. Cu/Zn superoxide dismutase

Down regulation of Cu/Zn superoxide dismutase in neuronal cell lines has been shown to be associated with increased apoptosis (Troy et al., 1996). Cu/Zn superoxide dismutase is thus an important endogenous antioxidant capable of neutralising free radicals.

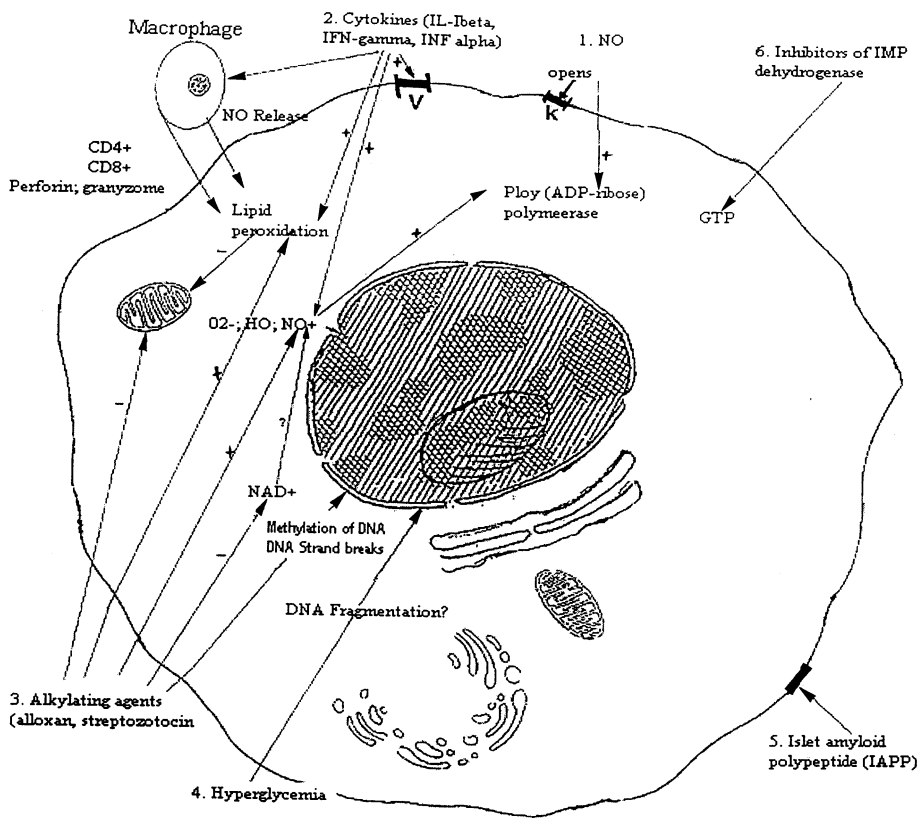

Fig. 1. Schematic diagram showing hypothetical pathways by which nitric oxide (NO), Cytokines, alkylating agents, hyperglycaemia, islet amyloid polypeptide (IAPP) and inhibitors of IMP dehydrogenase induce neuronal pancreatic beta cell death. k = potassium channel; v = low voltage-activated calcium channel; (+) = stimulation; (−) inhibition.

2.2.3. Glutathione

Depletion of glutathione is associated with increased brain level of NOS and a significant increase in neuronal apoptosis (Heales et al., 1996). Gluthathione peroxidase and glutathione reductase maintains the integrity of cells by neutralising free radicals. They are also important endogenous antioxidants.

2.2.4. Neurotrophins

Neurotrophins such as brain-derived neurotrophic factor (BDNF) prevent glutamate neurotoxicity via TrkB neurotrophic receptors (Akaike and Kume, 1998).

2.2.5. Growth factors

Growth factors such as neural growth factors (NGF) insulin growth factors (IGF) and basic fibroblast growth factor (BFGF) prevent NO-induced neuronal cell death. They can also promote cell survival by acting on phosphatidylinositol 3-kinase (PI3-kinase) and its downstream target, serine-thrionine kinase Akt. IGF can also prevent apoptosis and caspase-3 like activation induced by NO donors (Matsuzaki et al., 1999). BFGF inhibits changes in the level of Bcl-2 and Bax protein, which are components of the cascade of NO-induced apoptosis (Tamatani et al., 1998).

2.2.6. Neuroglial secretions

Astrocytes have been shown to produce substances capable of protecting neurons from damage caused by reactive oxygen and nitrogen species (Tanaka et al., 1999). Neuroglial cells participate in the metabolic activities of neurons and hence play important role in their survival and protection against noxious substances from the internal and external milieu.

2.2.7. Vitamins

2.2.7.1. Vitamin B12 analogues. Glutamate cytotoxicity on neurons can be prevented by exposure to methycobalamin, an analog of vitamin B12. Moreover, glutamate neurotoxicity was also prevented by chronic exposure of neurons to S-adenosylmethionine, which is formed in the metabolic pathway of methylcobalamin (Akaike et al., 1993).

2.2.7.2. Vitamin E. An antioxidant has been shown to protect neurons from the toxic effects of NO in a dose-dependent manner (Tagami et al., 1998).

2.2.8. 6R-tetrahydrobiopterin

6R-tetrahydrobiopterin is capable of enhancing neuronal survival. This action is exerted through the activation of calcium channels. This mechanism maintains optimum calcium levels (Koshimura et al., 1999). 6R-tetrahydrobiopterin also acts in protecting neural cells against NO damage by generating superoxide (Koshimura et al., 2000).

2.2.9. Cobalt

There are reports that showed that cobalt (a general voltage-gated calcium channel blockers) can prevent NO-induced motoneuronal cell death in vitro (Kaal et al., 1999).

2.2.10. Voltage-sensitive calcium blockers (e.g. amlodipine)

Agents that block voltage-sensitive calcium channels prevent neuronal apoptosis by preventing excessive calcium influx into neurons (Mason et al., 1999).

2.2.11. Melatonin

Melatonin detoxifies hydroxyl radicals as well as peroxyl radicals, peroxynitrite anion, NO and singlet O_2. In addition to this melatonin stimulates antioxidant enzymes including superoxide dismutase, glutathione peroxidase and glutathione reductase (Reiter et al., 1999). Melatonin is able to achieve all of these functions because it crosses the blood–brain barrier easily.

2.2.12. 1-S-R-daurisoline (DS)

A group of scientists have shown that low concentrations of DS inhibited glutamate neurotoxicity and increased cell viability in hippocampal neurons. DS prevent neural death by inhibiting glutamate-triggered NO generation (Liu et al., 1999).

2.2.13. Antibiotics

It has been shown that tetracylines reduced the level of interleukin-1 beta converting enzyme, caspase, which is produced in microglia after ischaemia. The lipid soluble varieties of tetracycline such as doxycycline and minocycline inhibit inflammation and are thus neuroprotective agents (Yrjanheikki et al., 1998).

2.2.14. Lubeluzole

Lubeluzole, an agent used to treat experimental and clinical ischaemia has a unique neuroprotective ability. It prevent neutral destruction by directly modulating the pathway of NO (Maiese et al., 1997)

3. Pancreatic beta cell death

The pancreatic beta cell is subjected to various internal and environmental toxins in addition to its programmed cell death. These toxic agents include free radicals like H_2O_2, O_2, cytokines, hyperglycaemia, islet amyloid polypeptide and immunosuppressive drugs that inhibit inositol monophosphate (IMP). The major cytokines that play direct or indirect roles in pancreatic beta cells destruction include, tumour necrosis factor alpha (TNF-α), interleukin-1-beta (IL-1-β) and interferon-gamma (IFN-γ). The mechanism by which these cytokines exert their effect has been a subject of intense research in the past few years. The aim of this review is to present a synopsis of the various agents responsible for pancreatic beta cell destruction and examine the ways in which this destruction can be prevented or suppressed.

3.1. Substances causing pancreatic beta cell death

3.1.1. Nitric oxide

Nitric oxide is a primary toxic effector molecule in the lysis of islet cells by inflammatory macrophages. This role is evident by the fact that *N*-nitro-L-Arginine-Methylester (NAME) an inhibitor of endothelial and macrophage nitric oxide synthase partially suppressed the development of diabetes mellitus in low-dose streptozotocin-induced diabetes model (Kolb et al., 1991). The beta cell dysfunction caused by nitric oxide is said to be associated with Type 1 diabetes (Inada et al., 1995). One of the possible mechanism of action of nitric oxide in pancreatic beta cell is the activation of the poly (ADP-ribose) synthetase that results in the decrease of NAD content, leading to an eventual cell death (Radons et al., 1994; Inada et al., 1995). Other roles attributed to nitric oxide in beta cell destruction include the mediation of blood flow dysfunction in hyperglycaemia-induced deficiency in microcirculation (Moldovan et al., 1996).

How does nitric oxide exert its destructive role on pancreatic beta cell? Some reports (Krippeit-Drews et al., 1995) have shown that nitric oxide causes beta cell dysfunction in diabetes by influencing the activity of ionic channels. For example it has been shown that nitric oxide opens K^+ channels through suppression of phosphofructokinase activity, this in turn inhibits glucose-induced insulin release in pancreatic beta cells (Tsumura et al., 1994).

Other investigators (Kaneto et al., 1995) suggested that exogenously and endogenously generated nitric oxide can bring about apoptosis of isolated rat beta cell by inducing the cleavage of DNA into nucleosomal fragments of 180–200 bp, nuclear shrinkage, chromatin condensation and apoptotic body formation.

Although, it has been said that nitric oxide inhibits insulin secretion and causes pancreatic beta cell death, nitric oxide can also stimulate insulin secretion at low micromolar concentration by de-energizing mitochondria and thereby triggering mitochondrial calcium release (Laffranchi et al., 1995).

3.1.2. Cytokines

Cytokines released by both T lymphocytes and activated macrophages, particularly, interleukin-1-beta (IL-1-β) have been shown to play a role, as immunologically effector molecules, in the inhibition of insulin secretion from pancreatic beta cells and induce beta cell destruction (McDaniel et al., 1996). Because of this cytokines, especially, IL-1-β have been implicated in the pathogenesis of insulin-dependent diabetes mellitus (Ma et al., 1996). Cytokines including tumour necrosis fac-

tor-alpha (TNF-α) and interferon-gamma (IFN-γ) have also been implicated in the pathogenesis of pancreatic beta cell death (Yamada et al., 1993). It was suggested that IL-1-β molecule inhibits insulin secretion and also causes subsequent beta cell death.

The IL-1-β-induced inhibition of insulin secretion is said to be dependent on the metabolism of L-arginine to nitric oxide. This is evident from the report which showed that NG-monomethylarginine, a competitive inhibitor of L-arginine-dependent enzyme NO synthase completely prevented IL-1-β -induced inhibition of glucose-stimulated insulin secretion as well as nitrite production by islet cells (Corbett et al., 1991; Flodstrom et al., 1999). Reports by many other investigators also support the view that cytokines do not contribute directly to the cytotoxic activity of activated macrophages towards the isolated islet cells (Andersen et al., 1966; Kroncke et al., 1991). They, however, act through effector molecules like NO. IFN-γ is a potential mediator of beta cell death. IFN-γ induce pancreatic cell death by activating caspase (Stephens et al., 1999), a substance implicated in the destruction of pancreatic islet death.

How does the cytokine-induced beta cell destruction occur? Cytokine-induced beta cell destruction is said to be mediated by inducing the production of oxygen free radicals, lipid peroxidation and aldehyde production. In these processes, malondialdehyde (MDA) has been shown to be one of the cytotoxic mediators of cytokine-induced beta cell destruction (Rabinovitch et al., 1996). Moreover, cytokines like IL-1-β has been shown to increase the cytosolic levels of nonesterified arachidonate as measured by isotope dilution spectrophotometry (Ma et al., 1996; Bleich et al., 1998; Zhou et al., 1998). This effect is suppressed by NG-monomethyl-arginine (NMMA) and mimicked by nitric oxide releasing compound 3-morpholinosydnonimine. All of these indicate that toxic arachidonic substrates are secreted during treatment of beta cells with IL-1-β.

In addition to the ability of cytokines to activate nitric oxide production in macrophages, cytokines have also been shown to activate nuclear transcription factor (NF-κB) in human pancreatic islets (Flodstrom et al., 1996). The activation of NF-κB factor has been shown to be a necessary but not sufficient signal for cytokine-induced iNOS expression in human islets of Langerhans (Flodstrom et al., 1996). In another report it was demonstrated that cytokine-induce beta cell death is mediated through low voltage-activated Ca^{2+} current (Zhou et al., 1998; Wang et al., 1999).

Antagonists of low voltage-activated Ca^{2+} can prevent the elevation of basal Ca^{2+} and DNA fragmentation caused by cytokines.

There are other views as to how pancreatic beta cell death is mediated by the immune system. Activated macrophages secrete IL-12, which stimulates CD4+ cells. This in turn secretes IFN-γ and IL-2. IFN-γ activates other resting macrophages to produce IL-1 β, TNF-α and free radicals, all of which are toxic to the beta cell. The IL-2 and other cytokines produced will thereafter promote the migration of CD8+ cells towards the target cells which are destroyed by perforin and granyzyme released by CD8+ cells (Fig. 1). CD4+ and CD8+ T cells also destroy pancreatic beta cells by stimulating Fas-dependent cytotoxicity (Giordano et al., 1998). Cytokines do not only inhibit insulin secretion, they also cause significant DNA strand breakage (Dunger et al., 1996). Recent reports showed that IL-1 β can disproportionately elevate proinsulin levels in pretype 1 diabetic patients when given in combination with IFN-γ (Holtens et al., 1999). This phenomenon is said to occur independently of beta cell death.

Substrates like D-glucose have been shown to potentiate the action of cytokines in the generation of nitric oxide required for beta cell destruction (Andersen et al., 1966).

Some reports indicated that nitric oxide might not be the major mediator of cytotoxic effects in human pancreatic islets (Eizirik et al., 1994a; Rabinovitch et al., 1994; Laffranchi and Spinas, 1997). This is because cytokines can stimulate other molecules, which are toxic to pancreatic beta cell (Fig. 1). In view of the toxic effect of cytokines on beta cells, cytokines have been implicated in the pathogenesis of insulin dependent diabetes mellitus (Sjoholm, 1999).

3.1.3. Alkylating agents

Alkylating agents including streptozotocin (STZ), alloxan (ALL), *N*-methyl-nitrosourea (MNU), *N*-ethyl-*N*-nitrosourea (ENU), Methyl-methanesulphonate (MMS) and ethylmethane-sulphonate (EMS) have all been shown to cause beta cell destruction in experimental animals (Delaney et al., 1995). Of all the six agents listed above, STZ and ALL are the most commonly used agents for the selective destruction of pancreatic beta cells in experimental diabetes. STZ is the more selective and more widely used of the two.

Streptozotocin (STZ) is a derivative of the fungus Streptomyces achromogenes. It was discovered in 1960 during a search for new anti-fungal agents (Vaura et al., 1959). During the investigations on the anti-fungal action of STZ, it was discovered that STZ also has anti-neoplastic, antibiotic and diabetogenic properties (Rakieten et al., 1963). Since then, either single or multiple subdiabetogenic injections of STZ have been used to induce diabetes mellitus. STZ has now become one of the most commonly used agents in studies on experimental diabetes. Many actions have been attributed to STZ. These changes may be similar to what has been described for the action of alloxan, which include damage to pancreatic beta cell membrane (Bhattacharya, 1954) and the depletion of intracellular NAD in islet cells (Schein et al., 1973). The effect of STZ on intracellular NAD is evident from the fact that STZ-induced diabetes can be prevented by the administration of nicotinamide (Hu et al., 1996). In addition to these, STZ has also been shown to induce DNA strand breaks and methylation in pancreatic islet cells (Uchigata et al., 1982; Matkovics et al., 1997/98). Recent result shows that nitric oxide is formed during the intracellular degradation of STZ (Kroncke et al., 1995).

A number of investigators have also shown that superoxide (O_2^-) anion, hydrogen peroxide (H_2O_2) and hydroxyl radical ($HO^{\cdot}$) play important role in the induction of neurovascular dysfunctions in diabetes mellitus (Cameron and Cotter, 1995). A number of investigators including, Okhuwa et al. (1995) demonstrated that hydroxyl radicals are the main factors involved in oxidative stress leading to the eventual cell death in STZ-induced diabetes mellitus. In addition to all of these changes caused by STZ, lipid peroxidation is said to increase which in turn leads to damage of cellular and mitochondrial membranes when beta cells are incubated with STZ (Matkovics et al., 1982).

STZ, MNU and ENU all release nitric oxide into the incubation medium. However, cyclic GMP, MMS and EMS, which do not have the nitroso group, did not show evidence of nitric oxide release (Delaney et al., 1995). This shows that NO release is not a function of all of the alkylating agents. The alkylating agents with the exception of alloxan, decreased glucose-stimulated insulin secretion. This observation suggests that nitric oxide release was not necessary for the inhibition of insulin secretion.

3.1.4. Hyperglycaemia

High glucose level has been shown to be toxic to pancreatic beta cell. Exposure of islets from diabetes-prone Psammomys obesus to high glucose levels resulted in a dose-dependent beta-cell DNA fragmentation (Donath et al., 1999). This observation appeared to be confined only to genetically predisposed animals since high glucose levels did not induce DNA fragmentation in normal rat islets (Fig. 1).

3.1.5. Islet amyloid polypeptide (IAPP)

Small aggregates of IAPP can cause membrane instability (Fig. 1) and induced vacuolisation and subsequent cell death in mouse and human islet cells (Janciauskiene and Ahren, 1998; Janson et al., 1999).

3.1.6. Inhibitors of Inositol Monophosphate dehydrogenase

New generations of immunosuppressive agents like mycophenolic (MPA) acid and mizoribine have been shown to deplete cellular GTP. Cell number, DNA and protein contents and metabolic viability were decreased after treatment of HIT cells with clinical concentrations of MPA (Li et al., 1998).

3.2. Prevention of pancreatic beta cell death

3.2.1. Nicotinamide

Nicotinamide, otherwise known as niacinamide or vitamin B3. Nicotinamide has been shown to decrease nitric oxide production and partially protects human pancreatic islet cells against the suppressive effects of a combination of cytokines (Eizirik et al., 1994b; Andersen et al., 1994). Nicotinamide can protect macrophage and interleukin-1-beta mediated beta cell damage in animal models of insulin-dependent diabetes mellitus (Andersen et al., 1994). Nicotinamide acts by suppressing poly (ADP-ribose) polymerase, thereby decreasing the consumption of NAD+ (Kolb and Burkart, 1999). This in turn leads to the modification of the cell death pathways.

3.2.2. Heat shock

Heat shock at 43°C for 90 min reduced cell death from nitric oxide by 80% and from streptozotocin by 90%. The occurrence of DNA strand break, a major early consequence of exposure to nitric oxide, reactive oxygen intermediates or streptozotocin was not suppressed by heat shock treatment. In contrast, the reduction of NAD+, a major cause of radical-induced pancreatic cell death was suppressed after heat shock treatment (Bellman et al., 1995).

3.2.3. Indigenous antioxidant enzymes

Many antioxidants are found in the internal *millieu* and they include gluthathione peroxidase, catalase and Cu/Zn superoxide dismutase. Overexpression of these antioxidant enzymes has been shown to suppress the NO and reactive oxygen-induced toxicity of pancreatic beta cell (Tiedge et al., 1999). Stimulation of these antioxidant enzymes could, therefore, prevent pancreatic beta cell death in a given clinical setting.

3.2.4. Alpha-tocopherol

This is commonly known as vitamin E and it is now widely used as an antioxidant. Burkart and associates (1995) showed that preincubation of pancreatic islet cells with alpha-tocopherol significantly improved their resistance to toxic doses of nitric oxide. They also showed that other anti-oxidants such as vitamin C or gluthathione-monoethyl ester did not offer the high degree of protection observed with α-tocopherol.

3.2.5. Copper

Copper ion is yet another agent capable of preventing the inhibitory effects of interleukin-1-beta on rat pancreatic islet function (Vinci et al., 1995). This was based on the assumption that Cu^{2+} is a necessary cofactor for both intramitochondrial enzymes involved in energy production and hydroxyl scavenger enzymes, two ways in which IL-1-β acts in the destruction of pancreatic beta cells.

3.2.6. Fusidic acid

Fusidic acid, a triterpenoid compound originally describe as an antimicrobial drug has been shown to protect islet beta cell destruction in type 1 diabetes mellitus. Although fusidic acid prevent beta cell destruction from the toxic effect of nitric oxide, no protection by fusidic acid was observed when beta cells were exposed to alkylating substances or oxygen radicals (Burkart et al., 1994).

3.2.7. Succinic acid

It has been shown that pancreatic islet cells exposed to IL-1-β in the presence of succinic acid monomethyl ester (SAM) have a higher insulin release in response to glucose as compared to islets cells incubated with IL-1-β alone. The beneficial effects of SAM were not accompanied by any reduction in IL-1-β-induced nitric oxide release nor inhibition of aconitase activity (Eizirik et al., 1994c). SAM was thought to improve beta cell function by increasing the ability of these cells to endure nitric oxide and partial inhibition of the Krebs cycle.

3.2.8. Glucocorticoids

Dexamethasone and probably other glucocorticoids can prevent IL-1-beta induced islet degeneration in experimental animals (Corbett et al., 1993). The ability of glucocorticoids to prevent pancreatic cell death is due to the suppressive action of glucocorticoids on macrophage and T lymphocyte activities.

3.2.9. Dihydroxylipoic acid

Burkart and associates (Burkart et al., 1993a) demonstrated that nitric oxide release from macrophages was suppressed in the presence of dihydroxylipoic acid. Dihydroxylipoic acid is also shown to scavenge free radicals. It was thus suggested that dihydroxylipoic acid possess anti-inflammatory properties with may protect target cells from oxygen radical attack.

3.2.10. Cyclosporin A

Cyclosporin A, a common immunosuppressive agent used in transplantation has been shown to suppress the toxicity of macrophages. Although, cyclosporin A does not improve the defense status of pancreatic beta cell against nitric oxide but rather inhibits the release of nitric oxide from activated macrophages (Burkart et al., 1993b).

3.2.11. Growth factors

Insulin-like growth factors I and II when given at a concentration of 100 ng/ml prevent pancreatic beta cell death in islets exposed to IL-1 β (5 ng/ml), TNF-γ (5 ng/ml) for 48 h (Mabley et al., 1997; Hill et al., 1999). Other reports, however, indicate that protection of cytokine-induced pancreatic cell death was only partially achieved with growth factors (Harrison et al., 1998). Growth factors can also prevent the Fas-mediated component of the cell destruction.

3.2.12. Gene therapy

The transfection of pancreatic islets with an anti-apopotic gene (bcl-2) have been shown to protect beta cells from cytokine-induced destruction (Iwahashi et al., 1998; Rabinovitch et al., 1999). Recent reports showed that mice lacking the poly (ADP-ribose) polymerase gene are resistant to pancreatic beta cell destruction and diabetes development induced by streptozotocin (Burkart et al., 1999).

3.3. Summary and future perspective

Nitric oxide appears to be the main culprit in both neural and pancreatic beta cell destruction and death. Toxic agents in both the environment and the internal *millieu* act through nitric oxide to kill neural and pancreatic beta cells. There is a strong evidence of a link between cytokines and NO-induced beta cell destruction and insulin dependent diabetes mellitus. A better understanding of the agents that can prevent beta cell death from these internal and environmental toxins could help in the prevention and treatment of diabetes mellitus. A better knowledge of neuroprotective drugs would help in the management of neurodenegerative diseases.

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