Biochimica et Biophysica Acta 1852 (2015) 980–991

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbadis

Expression of the kynurenine pathway enzymes in the pancreatic islet cells. Activation by cytokines and glucolipotoxicity

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ARTICLE INFO

Article history:
Received 29 October 2014
Received in revised form 29 January 2015
Accepted 3 February 2015
Available online 9 February 2015

Keywords:
Kynurenine pathway
IFN-γ
IL-1β
Glucolipotoxicity
β-cells
Non β-cells

ABSTRACT

The tryptophan/kynurenine pathway (TKP) is the main route of tryptophan degradation and generates several neuroactive and immunomodulatory metabolites. Experimental and clinical data have clearly established that besides fat, muscle and liver, pancreatic islet tissue itself is a site of inflammation during obesity and type 2 diabetes. Therefore it is conceivable that pancreatic islet exposure to increased levels of cytokines may induce upregulation of islet kynurenine metabolism in a way resembling that seen in the brain in many neurodegenerative disorders. Using normal rat islets and the INS-1 β-cell line, we have demonstrated for the first time that: 1/ only some TKP genes are constitutively expressed, both in β-cells as well as non β-cells; 2/ the regulatory enzyme indoleamine 2,3-dioxygenase (IDO1) is not constitutively expressed; 3/ IDO1 and kynurenine 3-monoxygenase (KMO) expression are potently activated by proinflammatory cytokines (IFN-γ, IL-1β) and glucolipotoxicity respectively, rather in β-cells than in non β-cells; 4/ Islet kynurenine/kynurenic acid production ratio is enhanced following IFN-γ and glucolipotoxicity; 5/ acute exposure to KYN potentiates glucose-induced insulin secretion by normal islets; and 6/ oxidative stress or glucocorticoid modulates TKP genes only marginally. Pancreatic islets may represent a new target tissue for inflammation and glucolipotoxicity to activate the TKP. Since inflammation is now recognized as a crucial mechanism in the development of the metabolic syndrome and more specifically at the islet level, it is needed to evaluate the potential induction of the TKP in the endocrine pancreas during obesity and/or diabetes and its relationship to the islet cell functional alterations.

1. Introduction

The tryptophan/kynurenine pathway (TKP) is the main route of tryptophan degradation in the human body and generates several neuroactive and immunomodulatory metabolites [1,2]. The first step in the TKP (Fig. 1) is characterized by the oxidation of tryptophan to N-formylkynurenine by the enzymes tryptophan 2,3-dioxygenase (TDO2) and indoleamine 2,3-dioxygenase (IDO1) [3–5]. TDO2 has been localized primarily in the liver, but has also been found in the brain, and is induced in the liver by tryptophan and corticosteroids [3,4]. IDO1, on the other hand, has been identified in several extrahepatic tissues, including the brain, and is up-regulated by cytokines and proinflammatory agents such as lipopolysaccharides [6], amyloid peptides [7], HIV proteins [8] and tumor cells [9]. However, its most potent stimulus is interferon-gamma (IFN-γ) [10]. The catabolite of tryptophan, N-formylkynurenine, is then hydrolyzed to form the first stable metabolite, kynurenine (KYN), by the action of kynurenine formamidase [11]. KYN can then be transformed by selected enzymatic activity to kynurenic acid (KYNA) and picolinic acid (PIC) side branches before complete catabolism to NAD^+. Importantly, not all tryptophan catabolism by the kynurenine pathway is directed towards the formation of intracellular NAD^+ [12]. A significant proportion of the tryptophan catabolites, KYN, KYNA, quinolinic acid (QUIN) and PIC leak out of the cell to exert as yet incompletely characterized biological activity. QUIN has been identified as a selective agonist, and KYNA as a selective antagonist, at the ubiquitous N-methyl-d-aspartate (NMDA) receptor in the central nervous system [13,14]. NMDA receptor activity has been implicated in both normal central nervous system

Abbreviations: CNS, central nervous system; INS-1, insulin secreting β-cell line originated from a rat insulinoma; GK rat, Goto–Kakizaki rat; NOD mouse, non-obese diabetic mouse; TKP, tryptophan/kynurenine pathway; Trp, tryptophan; KYN, kynurenine; 3HK, 3-OH-kynurenine; KYNA, kynurenic acid; QUIN, quinolinic acid; PIC, picolinic acid; AA, anthranilic acid; 3HAA, 3-OH-anthranilic acid; AMS, aminomuconic semialdehyde; ACMS, aminocarboxymuconate semialdehyde; NAD, nicotinamide adenine dinucleotide; IDO1, indoleamine 2,3-dioxygenase; TDO2, tryptophan 2,3-dioxygenase; KMO, kynurenine 3-monoxygenase; Kase, kynureninase; KAT1, KAT2, KAT3, KAT4, kynurenine aminotransferase 1-4; QPRT, quinolinatephosphoribosyltransferase; NAMPT, nicotinamidephosphoribosyltransferase; ACMSD, aminocarboxymuconatesemialdehyde decarboxylase; NMDA, N-methyl-d-aspartate; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; GPR35, G protein-coupled receptor 35; GSIS, glucose-stimulated insulin secretion

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http://dx.doi.org/10.1016/j.bbadis.2015.02.001
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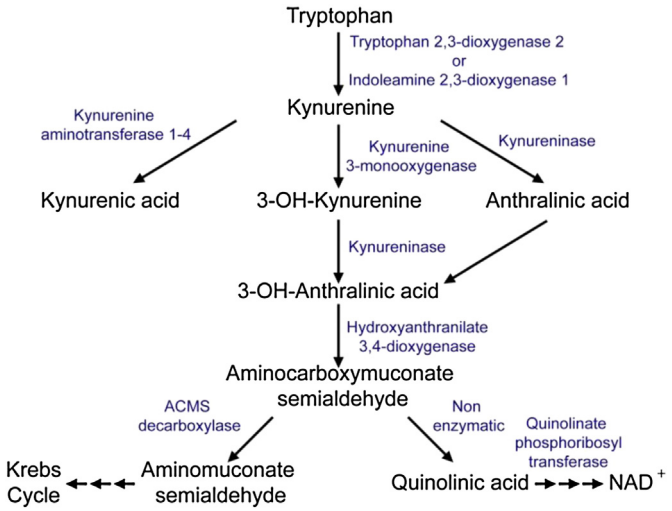

Fig. 1. The kynurenine pathway for the oxidative metabolism of tryptophan in mammalian cells.

development and inflammatory-mediated excitotoxicity. It is therefore likely that both QUIN and KYNA are actively involved in brain function and dysfunction [2,15–17]. Indeed, altered levels of TKP metabolites have been observed in multiple neuropsychiatric and neurodegenerative disorders [18–21] as well as in patients with affective disorders [22–26].

Importantly, several studies have shown that infections activate the TKP, which thereby appear to serve both as a direct defense mechanism and as a means of modulating the immune response [1,27,28].

Significant changes in TKP metabolism have also been reported in the brain, liver and kidney of rats with advancing age [29]. Although the cause or causes of these effects are still unknown, it is postulated that these changes are probably associated with the age-dependent increase in cellular and tissue damage that is mediated by increasing levels of free radicals and reactive oxygen species.

In neuronal cells, besides robust IDO1 induction by the proinflammatory cytokine interferon IFN-γ [30,31], it is not clear if pro-inflammatory cytokines affect expression of genes encoding other enzymes of the TKP.

Concerning pancreatic β-cells, it is known that they share with neuronal cells a large number of similarities. Different enzymes implicated in the synthesis of γ-aminobutyric acid or catecholamines, of specific cell-surface receptors for growth factors and amino acids, of specific intermediate filaments such as neurofilament, and of hormones such as thyrotropin-releasing hormone have been shown to be expressed in both neuronal and β-cells at different stages of their development [32]. Moreover, β-cells also resemble neurons by being electrically excitable and by responding to hormonal stimuli and glucose by depolarization and exocytosis in a process similar to neurotransmitter release from synaptic vesicles [32]. It has been proposed that similarities between β and neuronal cells are related to the expression in both cell types of identical transcriptional activators such as the transcriptional activator NeuroD/B2 [32], and to the lack of expression in both cell types of specific transcriptional repressors such as NRSF/REST [33].

Experimental and clinical data have clearly established that besides fat, muscle and liver, pancreatic islet tissue itself is a site of inflammation during obesity and type 2 diabetes [34]. Therefore it is conceivable that in parallel to the high free fatty acids and glucose levels, pancreatic islet exposure to increased levels of cytokines may induce dysregulation of islet TKP in a way resembling that seen in the brain in many neurodegenerative disorders. Recently, IDO1 expression was detected for the first time in human adipose tissue and found increased in adipose and liver tissues from obese patients in conjunction with obesity-induced low-grade inflammation [35].

While one study has reported a remarkable induction of IDO1 transcripts in human islets in response to cytokine-mediated inflammation [36], expression or functionality of genes encoding downstream enzymes in the TKP have never been investigated extensively in pancreatic endocrine cells. Since it is recognized that the TKP is cell-type dependent and can be controlled locally or systematically by different stimuli [37], it is of major importance to study the TKP in pancreatic islets, both in basal normal situation and in response to factors which might contribute to impair pancreatic islet cells such as exposure to cytokines, oxidative stress, high free fatty acids and high glucose levels. In the present study, we investigated if transcripts encoding enzymes in the TKP can be detected in normal rodent islet cells, and if their relative abundances are modulated by IFN-γ, IL-1β, glucolipotoxicity, ROS exposure or glucocorticoids.

2. Materials and methods

2.1. Materials

Cell culture medium was from Lonza, Montigny-Le-Bretonneux, France. Fetal bovine serum was from Biowest, Nuaillé, France. Cytokines were from Millipore, Fontenay sous Bois, France. Fatty-acid free BSA powders were from Roche Applied Science, Meylan, France. Apo-ONE® Homogenous Caspase-3/7 Assay kit was from Promega, Charbonnieres, France. RNA isolation kit was from Qiagen, Courtaboeuf, France. Reverse transcriptase kit was from Invitrogene, Saint Aubin, France. Real-time PCR kit was from Roche Applied Science, Meylan, France. All western blot materials were from BioRad, Marnes-la-Coquette, France. Rat insulin ELISA kit was from Alpco, Eurobio, Les Ulis, France. All solvents were from VWR, Fontenay-sous-Bois, France or Fisher Scientific, Illkirch, France, and all other chemicals used were from Sigma-Aldrich, Saint-Quentin Fallavier, France.

2.2. Rat islet isolation

Isolated islets were obtained from 10 to 13 week old male Wistar rats from our local colony bred in accordance with accepted standards of animal care as established by the French National Centre for Scientific Research. Pancreatic islets were isolated by collagenase digestion and Ficoll gradient purification. Islets were handpicked under a stereomicroscope as previously described [38].

2.3. Rat islet and β-cell line culture conditions

Rat islets were cultured in 2 ml of RPMI 1640 containing 10 mM glucose and supplemented with penicillin-streptomycin (500 U/ml), 2 mM L-glutamine, and 10% heat-inactivated fetal bovine serum. Islets were maintained free floating at 37 °C in a humidified atmosphere of 95%O_2–5%CO_2 until initiation of experiments.

Rat insulinoma INS-1 cells (clone 368), kindly provided by Merck-Serono, were grown in the same RPMI 1640 medium as described above and further supplemented with 50 μM 2-mercaptoethanol. Cells were plated for 48 h in 96-well plates (5×10^4 cells/well) for MTT assay and caspase 3/7 activity, in 12-well plates (5×10^5 cells/well) for insulin secretion.

Batches of 100 islets or 10^6 INS-1 cells (in 6-wells plates) were incubated in the absence or presence of cytokines, namely 500 units/ml interleukin IL-1β or 2500 units/ml IFN-γ ( ). In oxidative stress experiments, islets or INS-1 cells were pretreated with 50 μM H_2O_2 alone for 30 min and then washed twice with PBS before islets were cultured for 48 h in RPMI 1640 medium. In glucolipotoxicity experiments, islets or INS-1 cells were cultured for 48 h with or without palmitate (0.4 mM) in the presence of 10 mM (control) or 20 mM (G20) glucose. Palmitate was administered to the islets or INS-1 cells as a conjugate with fatty-acid free BSA. Briefly, dried aliquots of palmitate in ethanol were dissolved in PBS containing 13% (*w*/*v*) BSA to obtain a 10 mM stock