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

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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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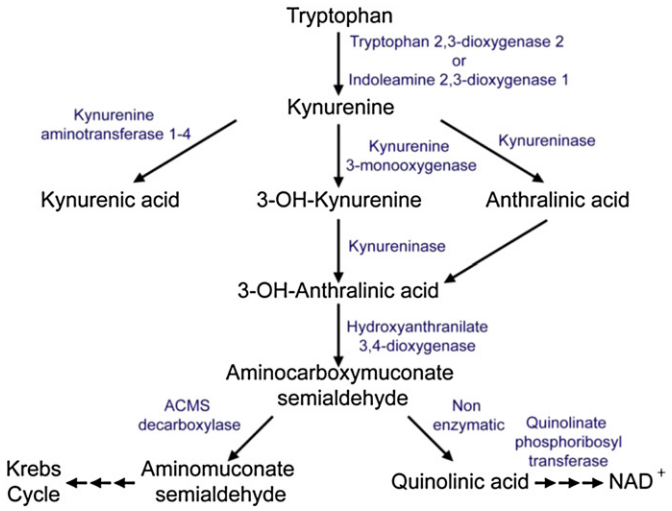

Fig. 1. The kynurenine pathway for the oxidative metabolism of tryptophan in mammalian cells.

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

solution. The molar ratio of palmitate to BSA was 5:1. The palmitate stock solution was diluted in RPMI 1640 medium supplemented with 10% FBS to obtain a 0.4 mM final concentration at a fixed concentration of 0.5% BSA.

2.4. Rat islet cell preparation and fluorescence-activated cell sorting of non β- and β-cells

Rat islets were trypsinized with trypsin (0.05%, Gibco, St Aubin, France) and digestion was stopped with cold Krebs–Ringer–bicarbonate–Hepes (KRBH) buffer—0.5% BSA (fatty-acid free) with 5.5 mM glucose. Cell suspensions were used for β-cell sorting. Using a FACSAriaII (Becton Dickinson, Pont De Claix, France), β-cells were distinguished from non β-cells and sorted based on their autofluorescence (FAD content) and cell size as previously described [39,40], resulting between our hands in a population with 94% (insulin-positive) β-cells as a mean. Data were analyzed using FACS DIVA analysis software (Becton Dickinson). Sorted β-cells and non β-cells were used for total RNA extractions or immunolabeling.

2.5. Immunolabeling on pancreas sections, isolated islets and isolated islet cells

Rat pancreas and isolated islets were fixed in Bouin solution and embedded in paraffin for section. Isolated islet cells were fixed by 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. Antigen retrieval was performed in a water bath for 15 min in a citrate buffer (pH 4) at 100 °C. The sections were blocked for 1 h with 10% normal goat serum in PBS. Multiple immunofluorescence labeling was performed with antibodies raised against IDO1 (1:50, Abcam), KMO (1:150, Abcam,), insulin (1:300, MP Biomedicals, Illkirch, France), glucagon (1:300, Boster, Interchim, Montluçon, France). Sections were then incubated with a mix of Alexa488-conjugated anti-rabbit (1:300, Invitrogen, St Aubin, France) and Alexa594-conjugated anti-mouse (1:300, Interchim, Montluçon, France) or Rhodamine X Red conjugated anti-Guinea pig (1:300, Vectorlabs, Eurobio, Les Ulis, France) secondary antibodies. Confocal images were acquired by Zeiss LSM 710 microscopy.

2.6. Quantitative PCR

Total RNA was extracted from rat islets, purified rat β-cells, non β-cells or INS-1 cells using the RNeasy mini kit. cDNA of each RNA sample was synthesized with MMLV (Moloney murine leukemia virus) reverse transcriptase using random hexamer primers. The primers used for PCR were derived from rat sequences and designed using OLIGO6 (the primer sets are described in Table 1). Real-time quantitative PCR amplification reactions were carried out in a LightCycler 480 detection system (Roche Applied Science) using the LightCycler SYBR Green 480 kit. Approximately 10 ng cDNA was used as the template for each reaction. All reactions were run in duplicate with no template control. The PCR conditions were: 95 °C for 10 min, followed by 40 cycles at 95 °C for 10 s, 60 °C for 10 s and 72 °C for 10 s. mRNA transcript levels of four housekeeping genes [encoding rpL19 (ribosomal protein L19), TBP (TATA-box binding protein), cyclophilin A and 18S] were assayed. Since similar results were obtained with the four housekeeping genes, only cyclophilin A was retained for normalization of other transcripts.

2.7. Western blotting

Equal amounts of proteins were separated by SDS/PAGE (12% gels) and then transblotted to PVDF membrane. The membranes were blocked with 5% (*w*/*v*) nonfat dry milk in TBS containing 0.05% (*v*/*v*) Tween 20. Blots were probed with a polyclonal anti-KMO antibody (1:500, Abcam), stripped and re-probed with a monoclonal anti-β-actin antibody (1:1000, Sigma-Aldrich). Immunoreactive bands were visualized by enhanced chemiluminescence with appropriate horseradish peroxidase-conjugated secondary antibody (Jackson ImmunoResearch Laboratories, Interchim).

Table 1
Transcripts analyzed by real-time PCR, gene symbols and primer sequences.

Target transcript	Gene	Polarity	Sequence (5′ → 3′)
IDO1	*INDO*	Sense	CAGTAGAGCATCAAGACCCGA
		Anti-sense	CCAACCAGACAAATATATGCGAAG
TDO	*TDO2*	Sense	CTGCTCTGCTTGTTCGATGAGAA
		Anti-sense	AGTGTGTCAATGTCCATAAGTGAGG
KMO	*KMO*	Sense	GGAGGAAGGGATACTCACGATG
		Anti-sense	CCCGTTCTTAGGTGGAATTGTC
KAT1	*CCBL1*	Sense	CAATGATGGCTGGAGGTTG
		Anti-sense	GTTGTTGGGTGTGTTGAGGA
KAT2	*AADAT*	Sense	ACATCATCTCCCTGGCTCCT
		Anti-sense	TGGTGCTTCCGTTCTCCAC
KAT3	*CCBL2*	Sense	GATTCAAAAATGCCAAACGAA
		Anti-sense	CCAAGATTCACCACAGAAGGA
KAT4	*GOT2*	Sense	TATGCCAAGAACATGGGCCT
		Anti-sense	GATCTTCAGCTGTGACTCCA
Kase	*KYNU*	Sense	GGCTGTTTTGTTGGCTTTGACCTA
		Anti-sense	CCGAACCATCCCACTAACGC
QPRT	*QPRT*	Sense	TGGACATGTAGCAGGCACGA
		Anti-sense	CAGGTCATAGCGGTGGCATT
ACMSD		Sense	CCAAAGGAATGGCCTGATCT
		Anti-sense	CTGAAGACCTTCCCGTCTTT
NAMPT		Sense	CAGAAGCCGAGTTCAACATC
		Anti-sense	TTTCACGGCATTCAAAGTAGG
TBP Sense		Sense	CGTGAATCTTGGCTGTAAACTTGA
		Anti-sense	GCTGCTAGTCTGGATTGTTCTTCA
Cyclophiline A		Sense	AACCCACCGTGTTCTTC
		Anti-sense	TGCCTTCTTTCACCTTCCC

2.8. Quantification of kynurenine and kynurenic acid

Levels of KYN and KYNA in islet culture media were determined using a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method. In short, culture medium samples were precipitated with methanol for Trp and KYN, or perchloric acid for KYNA, respectively. After centrifugation, the clear supernatant was injected into the LC-MS/MS system. Chromatographic separation was achieved on an Atlantis dC18 reverse phase column (C18, 3 μm, 20 × 4.6 mm (int. diam.)) and elution with (A) HCO_2H 0.1% (for positive mode on MS/MS), for 1 min and from A to (B) CH_3CN: A (40:60, *v*/*v*) in 2 min and for 2 min in B on a Agilent 1200 system with a quaternary pump. MS/MS was performed on an API 3200 LC-MS/MS spectrometer (Applied Biosystems/MDS Sciex, Villebon s Yvette, France) operated with a Turbo Ion Spray source in positive mode, looking for mass transition (MRM mode). Calibration ranges were 1–10,000 ng/ml for Trp, and 1–1000 ng/ml for KYN and KYNA, respectively.

2.9. Measurement of caspase 3/7 activity

Caspase 3/7 activity assays were performed using the Promega Apo-ONE® Homogenous Caspase-3/7 Assay kit, as previously described [41]. Briefly, lysis buffer containing the fluorigenic Z-DEVD-R110 (benzyloxycarbonyl-Asp-Glu-Val-Asp-rhodamine 110) substrate was added to each well and fluorescence was measured every 6 min over a 120 min period using a FLUOstar plate reader (MTX Lab Systems, Virginia, USA) set at 37 °C (ex:499 nm, and em:521 nm). Caspase 3/7 specific activity was expressed as the slope of the kinetic in arbitrary units. Each experimental condition tested was performed in triplicate.

2.10. Measurement of glucose-stimulated insulin secretion

At the end of the culture period, INS-1 cells were pre-incubated in KRBH (Krebs– Ringer/bicarbonate/Hepes) containing 0.2% fatty-acid-free BSA and 0.5 mM glucose for 1 h. Insulin secretion was measured

following a 30 min incubation in KRBH containing 0.2% defatted BSA with 0.5, 5 and 10 mM glucose.

Rat islets (batch of 6 islets) were preincubated in KRBH—0.2% BSA with 2.8 mM glucose for 1 h, followed by 90 min incubation in KRBH—0.2% BSA with 2.8 or 16.7 mM glucose to measure glucose-induced insulin secretion.

In some protocols, rat islet insulin release was measured during a perifusion procedure as previously described [38]. Eight freshly isolated islets at a time were allowed to attach on a polylysine-treated coverglass transferred to a perifusion chamber placed on the stage of an inverted microscope (Diaphot, Nikon, France). Cannulas feeding into the chamber were connected to a peristaltic pump and allowed a continuous perifusion of the islets at a flow rate of 1 ml/min with a 25 mM Hepes-buffered medium maintained at 37 °C, containing (in mM) 125 NaCl, 5.9 KCl, 1.28 CaCl2, 1.2 MgCl2, and 2.8 or 16.7 glucose (G2.8, G11), and 1 mg/ml BSA. The perifusion fluid was collected from the chamber at 60 s intervals and stored at −20 °C for insulin radioimmunoassay. The insulin concentration in the medium was determined by ELISA (Alpco) [41].

2.11. Statistical analysis

Results are expressed as means ± S.E.M. Significance was assessed using Student's unpaired and two-tailed *t* tests. $P > 0.05$ was considered significant.

3. Results

3.1. Detection of transcripts encoding TKP enzymes

In freshly isolated normal rat islets, all the TKP transcripts (TDO2, KMO, Kase, KAT1, KAT2, KAT3, KAT4, QPRT, NAMPT) were detected, except those of IDO1 and ACMSD. The levels of expression varied considerably across the different genes, with transcripts encoding KAT4 detected at the highest level (Fig. 2A).

Interestingly, after 48 h in culture, the hierarchy in the expression profile of the TKP genes was maintained (data not shown).

The INS-1 cells also exhibited differentially expressed TKP genes (Fig. 2B) and at variance with the primary rat islet cells, KMO and Kase transcripts remained undetected. Since it has been reported by others [31,42] that if one TKP enzyme is different, the production of the downstream KP metabolites can be totally different, one may conclude that INS-1 cells cannot be used as a blue-print of primary rat β-cells to investigate the TKP.

In separate experiments we also measured the TKP transcripts separately in β-cells and non β-cells isolated from normal rat islets maintained in a standard culture medium for 48 h (Fig. 2C). In the β-cell rich fraction (88% purity) of cells issued from islets, the levels of transcripts encoding IDO1 were undetectable, as they were in the non β-cell fraction (Fig. 2C). Transcripts encoding TDO2, KMO or KAT3 were similarly expressed in both cell populations (Fig. 2C), while those of Kase (0.46 fold, $p < 0.05$), KAT1 (1.7, fold), KAT2 (1.4 fold, $p < 0.05$), KAT4 (1.8 fold, $p < 0.05$), NAMPT (1.5 fold, $p < 0.05$) and QPRT (8.6 fold, $p < 0.05$) in the non β-cell population were significantly different compared to β-cell population. ACMSD transcripts were undetectable in β-cells as well as non β-cells.

3.2. Modulation of TKP transcript levels by proinflammatory cytokines

The potential effects of IFN-γ on TKP transcripts were investigated in cultured rat islets and INS-1 cells after a 48 h-exposure (Fig. 3).

In the INS-1 cells exposed to IFN-γ, the effect on IDO1 mRNA was the most remarkable (50 fold; $p < 0.05$) (Fig. 3A). KMO and Kase transcripts remained undetectable. QPRT, NAMPT and KAT3 were significantly up regulated (by 1.6, 1.6 and 1.7 fold; $p < 0.01$, $p < 0.01$ and $p < 0.05$ respectively). In the INS-1 cells exposed to IFN-γ, the TKP transcript changes

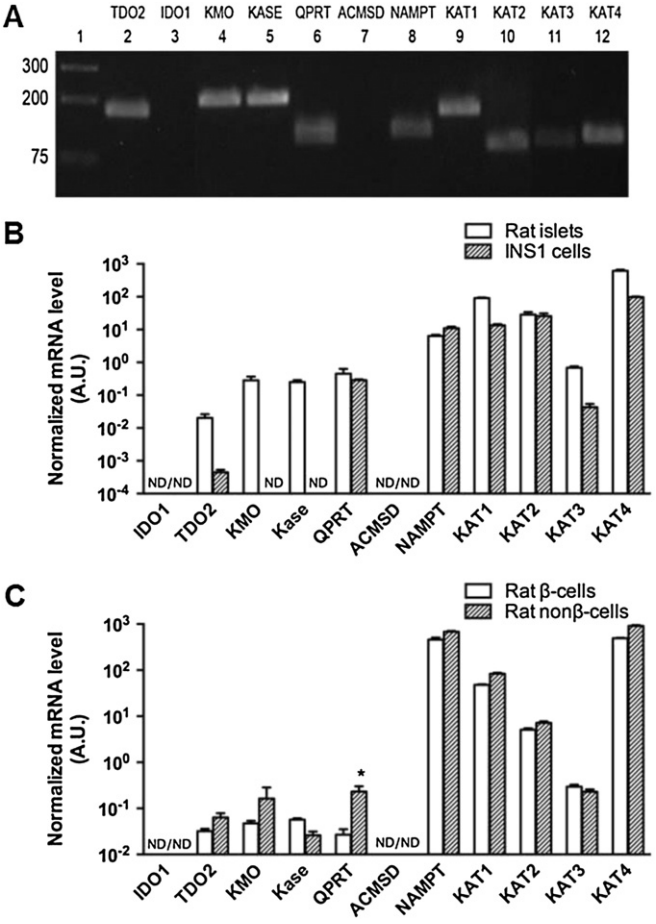

Fig. 2. Relative levels of transcripts encoding TKP enzymes in normal rat islets and INS-1 cells. (A) Reverse transcriptase-PCR analysis of TKP key enzymes. cDNAs were synthesized from fresh isolated rats islets. Amplifications of TDO2, IDO1, KMO, Kase, QPRT, ACMSD, NAMPT, and KAT1–4 are shown in lanes 2–12. Expected size of each PCR product was respectively: TDO2: 170 bp, IDO1: 152 bp, KMO: 190, KASE: 191 bp, QPRT: 100 bp, ACMSD 110 bp, NAMPT: 110 bp, KAT1: 161 bp, KAT2: 87 bp, KAT3: 94 bp, and KAT4: 102 bp. PCR product is not detected if reverse transcriptase is omitted from the reaction tube. However, IDO1 and ACMSD could be amplified in samples respectively obtained from IFNγ-treated rat islets and liver tissue (not shown). Therefore the absence of band in (A) experiments suggests that IDO1 and ACMSD expression levels in normal rat islets were undetectable under basal condition. (B) Relative levels of transcripts encoding TKP enzymes in normal rat islets (open bars) and INS-1 cells are shown (hatched bars). (C) Fresh isolated rat islets were trypsinized and cell populations sorted by FACS. Relative levels of TKP enzymes transcripts in sorted islets β-cells (open bars) and non β-cells (hatched bars) are shown. Data are expressed as means ± SEM of four independent experiments and quantified using $2^{-\Delta CT}$ method in log scale. * $p < 0.05$ as compared to rat β-cell group.

were observed in the presence of both increased cell apoptosis as estimated by increased caspase 3 activity (6.5 fold; $p < 0.01$) (Fig. 3D), and of impaired insulin release as estimated by decreased glucose-induced insulin secretion (Fig. 3E).

Also in rat islets treated with IFN-γ (2500 U/ml), the levels of transcripts encoding IDO1 were significantly increased (>140-fold compare to detection threshold; $p < 0.05$) compared to untreated cultures, which were inferior to detection threshold (Fig. 3B). Transcripts encoding TDO2, on the other hand, were slightly but not significantly down-regulated in IFN-γ treated islets as compared to controls (Fig. 3B). Transcripts encoding KMO, KAT1, KAT2, KAT4, Kase or QPRT were not significantly affected by the cytokine treatment (Fig. 3B), while those of KAT3 and NAMPT were significantly up regulated (by 1.7 and 1.6 fold; $p < 0.01$ and $p < 0.05$ respectively). These TKP transcript changes in the rat islets exposed to IFN-γ, were not correlated to impaired insulin release since glucose-induced insulin secretion remained normal (Fig. 3F).

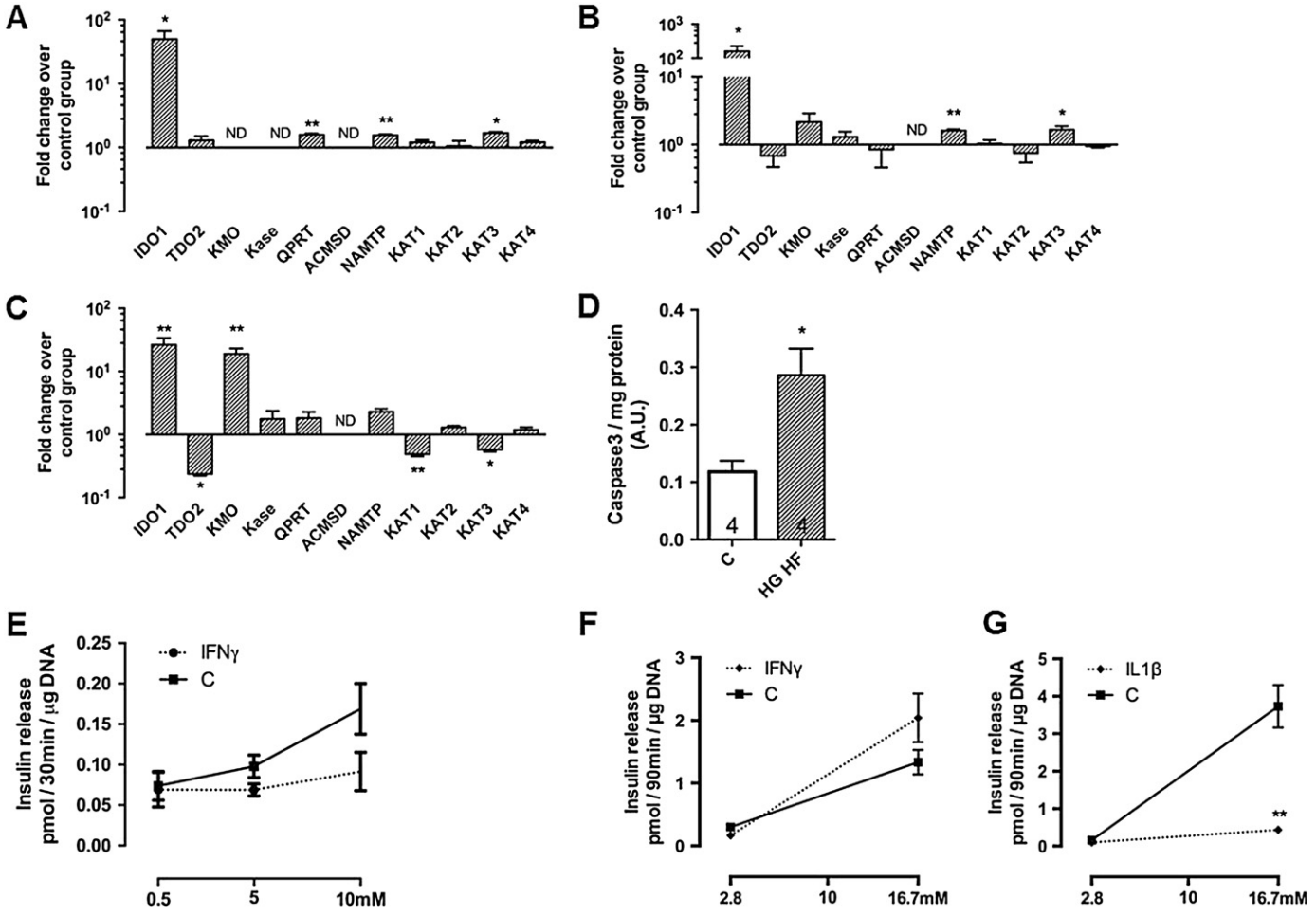

Fig. 3. Modulation of TKP transcript levels, insulin-cell survival and insulin secretion by proinflammatory cytokines. Changes in levels of transcripts encoding TKP enzymes in INS-1 cells challenged with IFN-γ (2500 U/ml) (A), rat islets challenged for 48 h with IFN-γ (2500 U/ml) (B) or IL-1β (100 U/ml) (C). Caspase 3 activity (D) and glucose-induced insulin secretion (E) by INS-1 cells challenged with IFN-γ (2500 U/ml). Glucose-induced insulin secretion by rat islets challenged with IFN-γ (2500 U/ml) for 48 hrs (F) or challenged with IL-1β (100 U/ml) for 48 h (G). Data are expressed as means ± SEM of four independent experiments. * $p < 0.05$, ** $p < 0.01$ as compared to related untreated control group (A,B,C,D) or to untreated islets at 2.8 mM glucose (G).

We also investigated the direct effect of IL-1β on rat islets after a 48 h-exposure. As indicated in Fig. 3C, 500U/ml IL-1β induced a robust expression of IDO1 (26 fold compared to detection threshold; $p < 0.01$), of KMO (19 fold; $p < 0.01$) and exerted some upregulating effect on Kase (1.8 fold; ns), NAMPT (2.3 fold, $p < 0.01$) and QPRT (1.8 fold; ns) expression. IL-1β also inhibited the expression of TDO2 (0.24fold; $p < 0.05$), KAT1 (0.49 fold; $p < 0.01$) and KAT3 (0.58 fold; $p < 0.05$). These TKP transcript changes in the rat islets exposed to IL-1β, were observed in the presence of impaired insulin release as estimated by decreased glucose-induced insulin secretion (Fig. 3G).

3.3. Modulation of TKP transcript levels by glucolipotoxicity

The potential effects of a 48 h-exposure to the combination high glucose (HG, 20 mM glucose)/high fat (HF, 0.4 mM palmitate) on TKP transcripts were investigated in INS-1 cells and cultured rat islets (Fig. 4).

In the INS-1 cells exposed to HGHF, IDO1, Kase transcripts and KMO transcripts remained undetectable. Transcripts encoding KAT2, KAT4, QPRT and NAMPT were not significantly affected by HGHF treatment (Fig. 4A), while those of KAT1 and KAT3 were decreased (by 0.6 and 0.1 fold; $p < 0.01$).

In INS-1 cells exposed to HGHF, the TKP transcript changes were observed in the presence of both increased cell apoptosis as estimated by increased caspase 3 activity (>2.4 fold; $p < 0.05$) (Fig. 4C), and of impaired insulin release as estimated by decreased glucose-induced insulin secretion (0.28 fold; $p < 0.01$ at G10) (Fig. 4D).

The TKP transcript pattern in rat islets treated with HGHF was different: the level of TDO2 transcripts was slightly but not significantly down-regulated; that of IDO1 transcripts remained undetected, and that of KMO transcripts was significantly increased (7.6 fold; $p < 0.05$) as compared to values in untreated cultured islets (Fig. 4B). Transcripts encoding KAT4, QPRT and NAMPT were modestly but significantly increased (by 1.5, 2.2 and 2.4 fold respectively; $p < 0.01$, $p < 0.05$ and $p < 0.05$ respectively) by HGHF treatment (Fig. 4B), while those of KAT1, KAT2 and KAT3 were modestly decreased (by 0.52, 0.5 and 0.51 fold respectively; $p < 0.01$ $p < 0.05$ and $p < 0.01$ respectively). These TKP transcript changes in the rat islets exposed to HGHF were observed in the presence of impaired insulin release as estimated by decreased glucose-induced insulin secretion (Fig. 4E).

3.4. Modulation of TKP transcript levels by oxidative stress

The potential effects of exposure to a calibrated oxidative stress (50 μM H_2O_2) on TKP transcripts were investigated in cultured rat islets and INS-1 cells (Fig. 5).

In the INS-1 cells exposed to H_2O_2, IDO1, KMO, Kase and ACMSD transcripts remained undetectable (Fig. 5A). Transcripts encoding TDO2, QPRT, NAMPT, KAT1, KAT2, KAT3 and KAT4 were not significantly changed (Fig. 5A). In the INS-1 cells exposed to H_2O_2, the TKP transcript

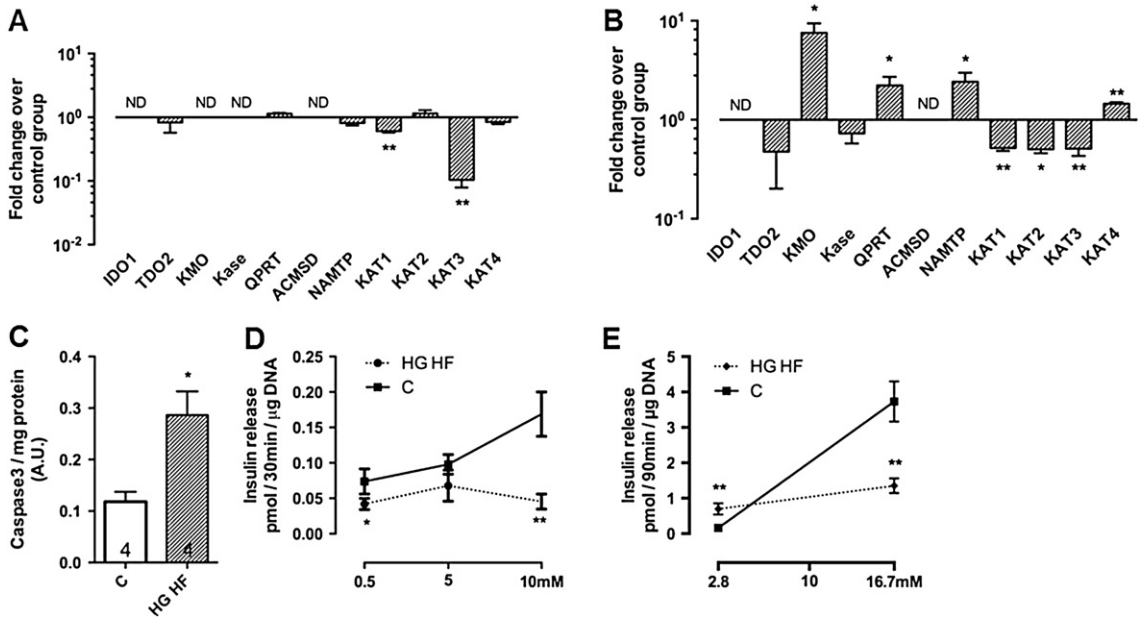

Fig. 4. Modulation of TKP transcript levels, insulin-cell survival and insulin secretion by glucolipotoxic environment. Changes in levels of transcripts encoding TKP enzymes in INS-1 cells (A) or rat islets (B) challenged with glucolipotoxic medium (HGHF) (20 m M glucose and 0.4 m M palmitate) during 48 h. Caspase 3 activity (C) and glucose-induced insulin secretion (D) by INS-1 cells challenged with HGHF. Glucose-induced insulin secretion by rat islets challenged with HGHF for 48 h (E). Data are expressed as means ± SEM of four independent experiments. * $p < 0.05$, ** $p < 0.01$ as compared to related untreated control group (A,B,C,D), to untreated INS1 cells at 0.5 mM glucose (D) or to untreated islets at 2.8 mM glucose (E).

changes were observed in the presence of increased cell apoptosis (1.7 fold; $p < 0.01$) (Fig. 5C), while the glucose-stimulated insulin release capacity remained unaffected (Fig. 5D).

In rat islets treated with H_2O_2, none of the TKP transcript levels was increased. Rather, they were globally down-regulated as compared to control levels (Fig. 5B), either non significantly (TDO2, KMO, Kase, QPRT) or significantly (KAT1, KAT3, KAT4: by 0.5, 0.77 and 0.7 fold respectively; $p < 0.01$). Transcripts encoding IDO1 remained undetectable. NAMPT and KAT2 transcript levels were not affected.

3.5. Modulation of TKP transcript levels by glucocorticoids

The potential effect of corticosterone on TKP transcripts was investigated in cultured rat islets after a 48 h-exposure (Fig. 6).

In rat islets treated with corticosterone (10 μM) the levels of transcripts encoding IDO1 were slightly induced (>1.8 fold compared to detection threshold; $p = 0.3$) (Fig. 6A) compared to untreated cultures which were inferior to detection threshold. Transcripts encoding TDO2, on the other hand, were slightly down-regulated

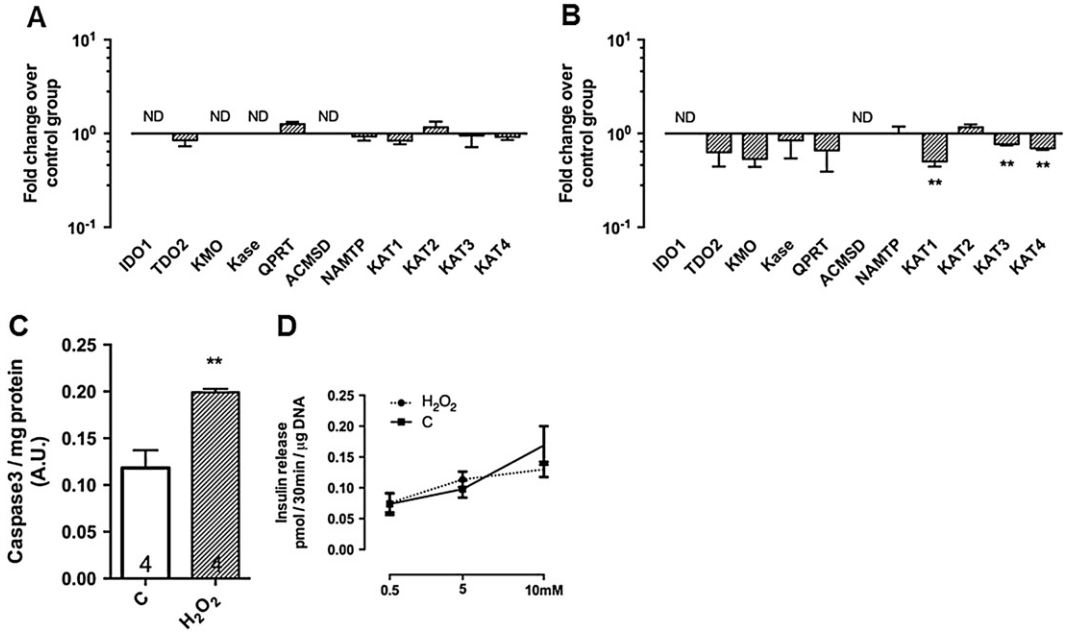

Fig. 5. Modulation of TKP transcript levels, insulin-cell survival and insulin secretion by oxidative stress. Changes in levels of transcripts encoding TKP enzymes in INS-1 cells (A) or rat islets (B) challenged with oxidative medium (50 μM H_2O_2) for 30 min, then washed and cultured for 48 h in normal medium. Caspase 3 activity (C) and glucose-induced insulin secretion (D) by INS-1 cells challenged with oxidative medium (50 μM H_2O_2) for 30 min, then washed and cultured for 48 h in normal medium. Data are expressed as means ± SEM of four independent experiments. * $p < 0.05$, ** $p < 0.01$ as compared to related untreated control group (A,B,C).

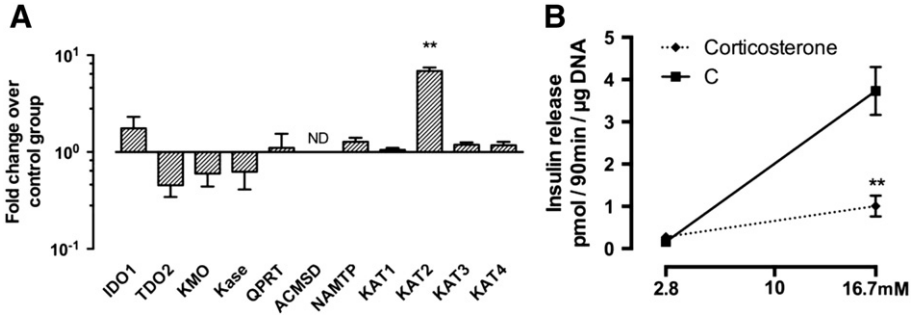

Fig. 6. Modulation of TKP transcript levels, insulin-cell survival and insulin secretion by glucocorticoid. Changes in levels of transcripts encoding TKP enzymes (A) and glucose-induced insulin secretion (B) in rat islets challenged with corticosterone (10 μM) for 48 h. Data are expressed as means ± SEM of four independent experiments. ** $p < 0.01$ as compared to related untreated control group (A) or to untreated islets at 2.8 mM glucose (B).

(while not significantly) in corticosterone treated islets as compared to controls (Fig. 6A). Transcripts encoding KMO, Kase, QPRT, NAMTP, KAT1, KAT3 and KAT4 were not significantly affected by corticosterone treatment (Fig. 6A), while those of KAT2 were significantly up regulated (by 6.9 fold; $p < 0.01$). ACMSD transcripts were undetectable. These TKP transcript changes in the rat islets exposed to corticosterone, were observed in the presence of impaired insulin release as estimated by decreased glucose-induced insulin secretion (Fig. 6B).

3.6. Modulation of TKP transcript levels by the combination cytokines + glucolipotoxicity

In additional experiments we measured the TKP transcripts separately in β-cells and non β-cells isolated from normal rat islets previously exposed during 48 h to the combination IFN-γ + HGHF (Fig. 7A).

In the β-cell rich fraction of cells issued from islets treated with the combination, the levels of transcripts encoding IDO1 were strongly and significantly induced (570 fold compared to detection threshold; $p < 0.05$) compared to untreated control β-cells (Fig. 7A). KMO transcripts were also significantly increased (>8 fold; $p < 0.05$) as compared to values in untreated β-cells (Fig. 7A). Transcripts encoding TDO2, Kase, KAT1, KAT2 or QPRT were not significantly affected (Fig. 7A), while those of NAMPT, KAT3 and KAT4 were modestly but significantly upregulated (by 2, 1.9 and 1.6 fold respectively; $p < 0.01$ $p < 0.05$ and $p < 0.01$ respectively).

In the non β-cell rich fraction of cells issued from islets treated with the combination, the pattern was not identical. While the levels of transcripts encoding IDO1 were also strongly and significantly increased

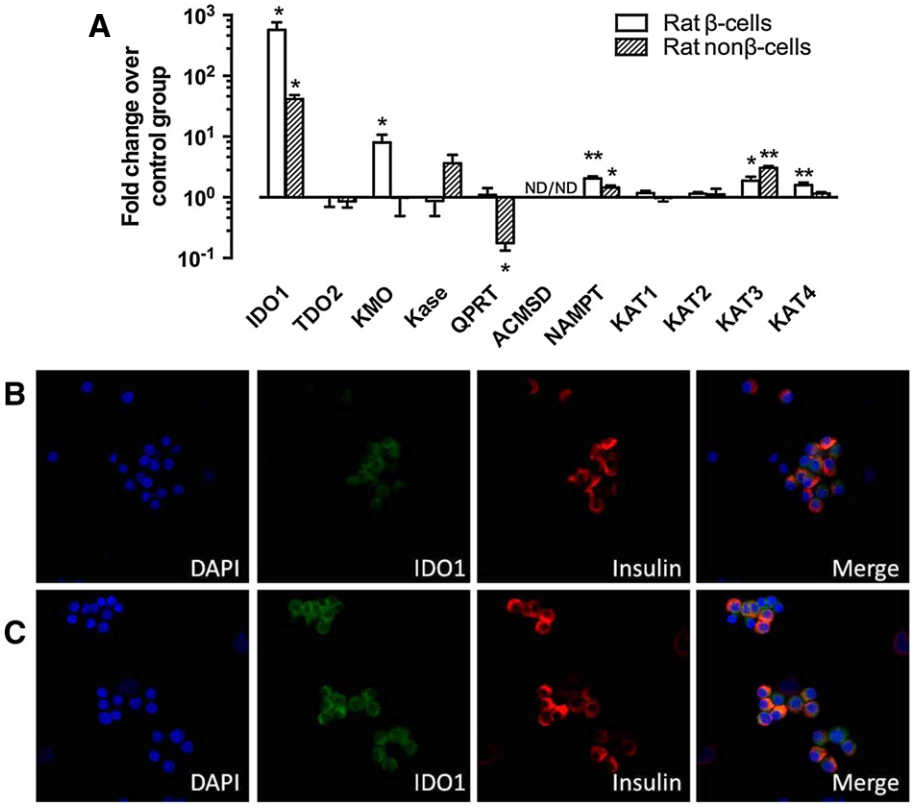

Fig. 7. TKP transcript levels in isolated rat β-cells and nonβ-cells exposed to the combination glucolipotoxic medium + IFN-γ, and IDO1 immunodetection in isolated rat β-cells. Freshly isolated rat islets were cultured in a combination of glucolipotoxic medium (HGHF) and IFN-γ (20 mM glucose, 0.4 mM palmitate and 2500 U/ml IFN-γ) for 48 h, then trypsinized and sorted by FACS into a β-cell fraction (open bars) and a non β-cell fraction (hatched bars). Changes in levels of transcripts encoding TKP enzymes were quantified in both populations (A). IDO1 immunodetection was performed in β-cell fractions obtained without (B) or after exposure of rat islets to IFN-γ treatment (C). Data in (A) are expressed as means ± SEM of four independent experiments. * $p < 0.05$, ** $p < 0.01$ as compared to related untreated control group.

(42 fold; $p < 0.05$) compared to untreated control non β-cells (Fig. 7A), KMO transcripts were not significantly modified (0.98 fold; $p = 0.96$) as compared to values in untreated non β-cells (Fig. 7A). Kase transcripts were slightly upregulated, but the difference as compared to control did not reach statistical significance (3.6 fold; $p = 0.055$). Transcripts encoding TDO2, KAT1, KAT2, and KAT4 were not significantly affected (Fig. 7A), while those of NAMPT and KAT3 were up-regulated (by 1.4 and 3 fold respectively; $p < 0.05$ and $p < 0.01$ respectively) and those of QPRT were down-regulated (by 0.18 fold; $p < 0.05$) (Fig. 7A). ACMSD transcripts were undetectable in both cell fractions (Fig. 7A).

3.7. IDO1 and KMO protein expression and localization in normal rat pancreas and islet cells

We were also unable to detect any IDO1 immunoreactivity in endocrine cells as well as while exocrine tissue in the basal normal situation (data not shown). In additional experiments, we analyzed IDO1 immunolocalization separately in β-cells isolated from normal rat islets previously exposed in vitro during 48 h to IFN-γ. Under these conditions only, all the β-cells exhibited a discrete IDO1 co-labeling (Fig. 7C).

Western blot analysis detected basal expression of KMO protein in rat islets cultured 48 h in normal medium (Fig. 8I) and showed significant increase of KMO protein in response to IL-1β (Fig. 8I). Immunofluorescence analysis performed on whole adult rat pancreatic sections demonstrated the presence of a strong KMO immunoreactivity in glucagon positive cell and a very faint immunoreactivity in insulin positive cells, while exocrine tissue was devoided of any staining (Fig. 8A). Fig. 8 also provides images of KMO staining at different ages (pancreas at neonatal age day 7; pancreatic bud at embryonic age day 13) (Figs. 8B and 8C respectively) and co-localization with glucagon (Figs. 8B and C). From these example images, it is clear that the highest intensity of KMO immunostaining under basal state, whatever the age, co-localizes with glucagon and thus occurred within the α-cells.

In the islets cultured 48 h in normal medium, KMO expression was maintained and KMO staining was mostly located in the α-cells, since it co-localized with glucagon rather than insulin (Fig. 8D). In the islets exposed to HGHF conditions, KMO immunoreactivity became strongly increased in the insulin positive cells (Fig. 8E).

In additional experiments we also analyzed KMO immunolocalization separately in β-cells and non β-cells isolated from normal rat islets previously exposed during 48 h to normal culture medium (Fig. 8G) or the combination IFN-γ + HGHF (Fig. 8H). Immunofluorescence analysis performed on β-cells and non β-cells purified from untreated cultured islets, showed that all the insulin-positive cells were negative for KMO immunoreactivity and the KMO-positive cells were insulin-negative (Fig. 8G) and glucagon-positive (data not shown). A strong increase in the number of KMO immunopositive cells was observed in response to IFN-γ + HGHF, that included both insulin immunoreactive β-cells ((Fig. 8H) and non β-cells (data not shown). However, KMO staining was the strongest in the insulin-negative cells (non β-cells) and milder in the insulin-positive cells (β-cells). In this respect, the response in purified islet cells paralleled the effects detected by immunofluorescence analysis in intact islets.

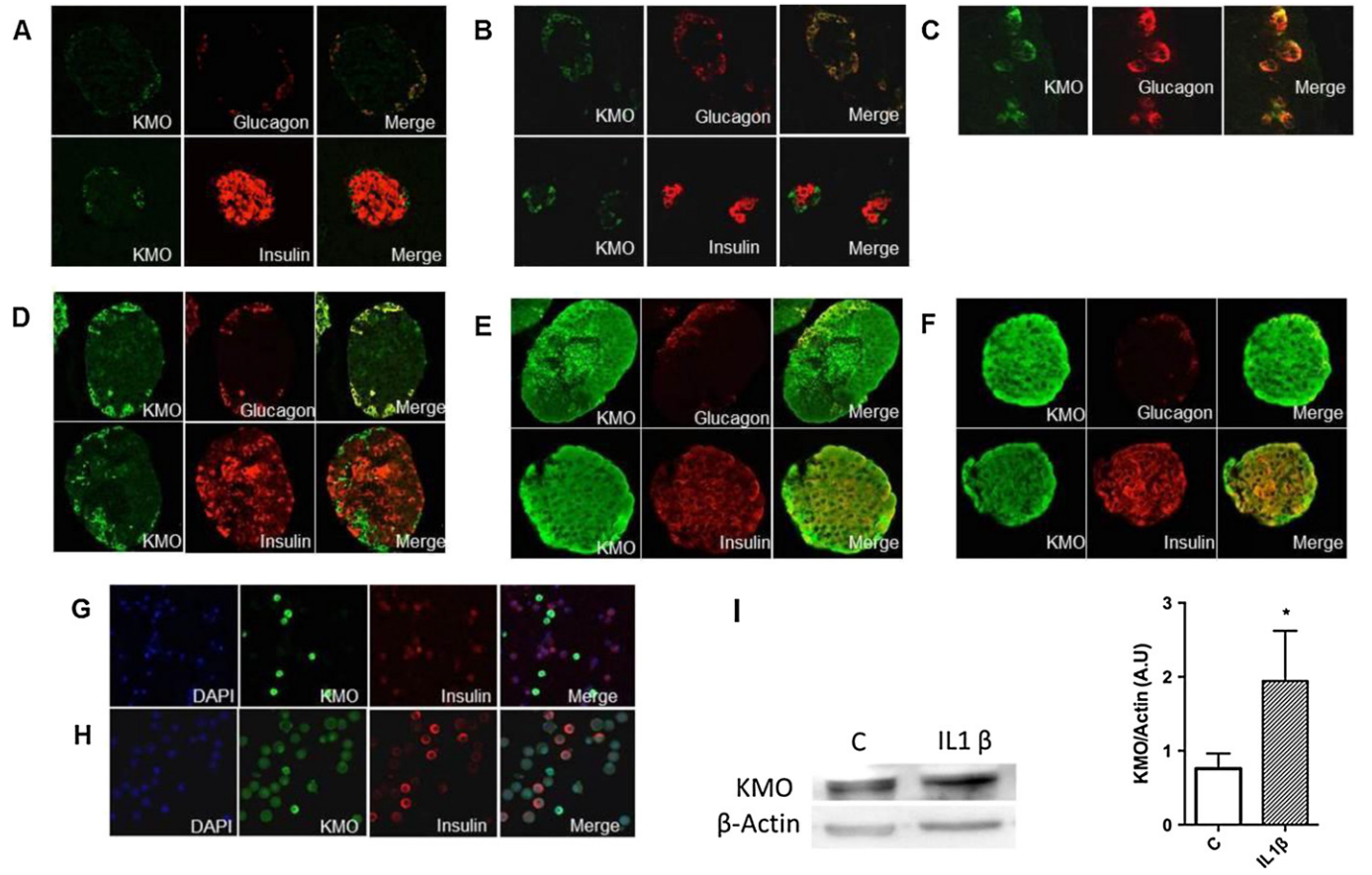

Fig. 8. KMO protein expression and immunolocalization. KMO immunolocalization was performed: 1/ in total pancreatic tissue from 12 week old normal rats (A), 7 day old newborn rats (B) and 13 day old rat fetuses (C). 2/ in rat islets after 48 h culture in normal medium (D), HGHF medium (20 mM glucose and 0.4 mM palmitate) (E), or the combination HGHF + IFN-γ (20 mM glucose, 0.4 mM palmitate and 2500 U/ml IFN-γ) (F); 3/ in isolated rat β-cells sorted from rat islets cultured for 48 h in normal medium (G), or the combination HGHF + IFN-γ (20 mM glucose, 0.4 mM palmitate and 2500 U/ml IFN-γ) (H). Western blot analysis for KMO protein expression (I) was performed in rat islets cultured for 48 h in normal medium (lane 1) or in medium containing 500 U/ml IL-1β (lane 2). Data in (I) are expressed as means ± SEM of four independent experiments. * $p < 0.05$ as compared to control group.

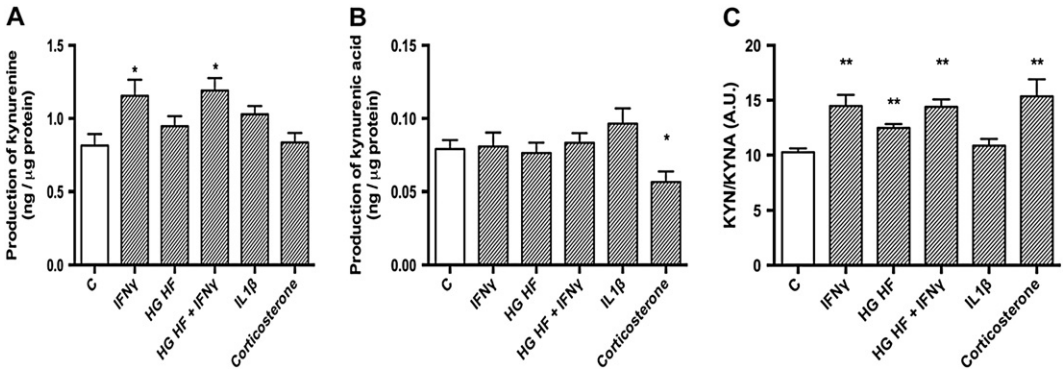

Fig. 9. KYN and KYNA production by normal rat islets exposed to various treatments in vitro. Groups of 200 normal rat islets were cultured for 48 h in medium under the indicated conditions. KYN (A) and KYNA (B) accumulations in the medium supernatants were measured by LC-MS/MS. Results were normalized to islet total protein content. In each condition, the KYN over KYNA ratio was calculated (C). Data are expressed as means ± SEM of four independent experiments. * $p < 0.05$ as compared to control group.

3.8. Modulation of islet KYN and KYNA release by the different treatments

To address potential functionality of the TKP in the cultured islets, we quantified the accumulation of KYN and KYNA in the islet culture media at the end of the 48 h culture period, after exposure to the various treatments (Fig. 9). First, levels of KYN and KYNA were detectable in medium from untreated cultured islets (Fig. 9). Notably, KYNA release represented only 10% of KYN release under this basal situation. Second, significantly ($p < 0.05$) higher levels were detected in media of islets treated with IFN-γ (by 1.4 fold) and the combination IFN-γ + HGHF (by 1.4 fold). HGHF or IL-1β had only a slight increasing effect which remained not statistically significant. Corticosterone did not affect KYN release (Fig. 9A). Concerning islet KYNA release, it was only significantly affected in response to corticosterone exposure (decreased by 0.7 fold; $p < 0.05$) (Fig. 9B). When islet KYN release was expressed as a function of islet KYNA release, it appeared to be clearly increased ($p < 0.01$) in response to IFN-γ, HGHF, IFN-γ + HGHF and corticosterone (Fig. 9C), but not to IL-1β.

3.9. Extracellular Trp, KYN and KYNA modulate GSIS by normal rat islets

Since we have shown that normal W rat islets spontaneous release significant levels of the TKP metabolites KYN and KYNA, it is reasonable to ask the question whether or not Trp, KYN and KYNA are able to modulate islet GSIS. To that aim, freshly isolated normal rat islets were perifused in the presence or absence of the metabolites. The kinetic of insulin release in the perifusion buffer is illustrated by Fig. 10. Cumulative values of insulin secretion during the glucose stimulation period (area under curve) are shown in the inset of Fig. 10. During perifusion with a submaximal stimulatory glucose concentration (11 mM), addition of 0.1 mM KYN significantly amplified the insulin release (2.2 fold, $p < 0.01$ compared to control group) (Fig. 10). 0.1 mM Trp or KYNA also amplified the GSIS by normal islets, but more modestly and the differences did not reach statistical significance (Fig. 10).

4. Discussion

We here report, for the first time, that rat pancreatic islet cells and the rat INS-1 β-cell line express detectable levels of transcripts encoding the different enzymes of the TKP. Importantly, this is associated with the spontaneous release of significant levels of the TKP metabolites KYN and KYNA by the rat islets, therefore suggesting some degree of functionality of the TKP in the rat islet cells in the basal situation. Substantial differences in the basal levels of expression across genes were also maintained during culture and likely reflect a stable and genuine genomic pattern.

Our results first show that IDO1 transcript and IDO1 protein are not expressed in the rat pancreatic islet under basal unstimulated condition, similarly to the human islet [36]. We demonstrated that IFN-γ alone or IL-1β strongly activate IDO1 mRNA accumulation in rat islets. By contrast, exposure to HGHF or H_2O_2 did not activate IDO expression, at least under our experimental setting which was nevertheless sufficient to deteriorate INS-1 cell survival and glucose-stimulated insulin secretion. Also in human islets, an effect of IFN-γ on IDO1 transcripts has been documented in microarray profiling experiments [36,43]. Concerning IDO1 protein expression, our FACS-purified cell experiments suggest that IFN-γ activates accumulation of IDO protein in both rat β-cells and non β-cells, but the specific immunostaining signal remained faint. More convincing to support the involvement of IDO1 activation following treatment with IFN-γ, is our finding that IFN-γ increased KYN release by the rat islets while KYNA release remained unchanged. Increased levels of TKP metabolites and increased levels of transcripts encoding IDO1 have been also reported following IFN-γ treatment of human fetal astrocytes [14], human skin fibroblast cultures [44] and in primary neurons and neuroblastoma cells [13]. Concerning IL-1β, a recent report demonstrated that it induced an up-regulation of IDO1 transcripts in human hippocampal progenitor cells [45], an observation consistent with our finding. In addition, our results show a faint IDO1 induction by corticosterone, as we detected a very tiny amount IDO1 amplicon in the corticosterone-treated samples compared

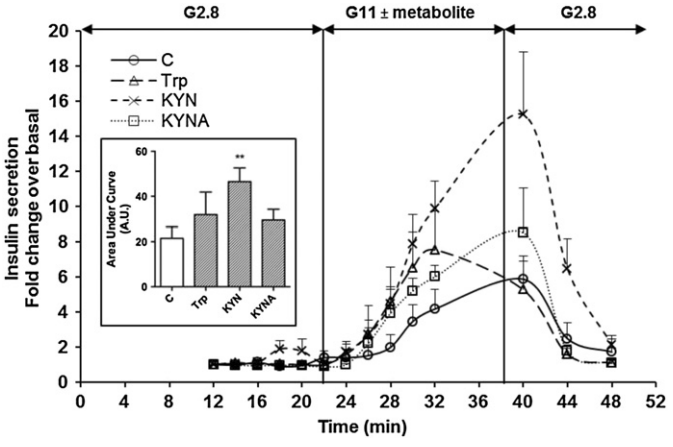

Fig. 10. Kinetics of glucose-induced insulin secretion by normal rat islets in response to Trp, KYN or KYNA. Groups of 30 normal rat islets were perifused with Hepes buffer containing 2.8 mM glucose (G2.8) or 11 m M glucose ± 100 μM metabolites as indicated. The secretion of insulin was normalized by the basal secretion at 2.8 mM glucose. Cumulative secretion values (AUC) during the stimulation period (22–40 min) are indicated in the inset graph. Data are means ± SEM of 7 experiments in each group. ** $p < 0.01$ compared with the control group (C).

to zero amplicon in the untreated group. However, due to high variability, the result did not reach statistical significance.

TDO2 transcripts and protein were moderately expressed in the rat pancreatic islet under basal unstimulated condition (as well as in the INS-1 cell line). IFN-γ, IL-1β, HGHF, or the combination IFN-γ + HGHF, induced a decrease of the level of TDO mRNA in rat islets, which became significant in response to IL-1β. Also in neuroblastoma cells, levels of transcripts encoding TDO2 were reduced by IFN-γ [13] and a switch between IDO1/TDO2 after IFN-γ stimulation has been previously described in human neurons [31]. Exposure to corticosterone did not activate islet TDO2 expression (either transcripts or protein), at variance with the effect reported in liver. Unfortunately, lack of a suitable antibody did not allow immunohistochemical studies of TDO2 protein.

In the KYNA branch of the TKP, four subtypes of KAT have been identified within the brain, with KAT2 being the most abundant in rat and human brain [46]. The four KAT enzymes were found constitutively expressed in rat pancreatic islets as well as in the INS-1 cells, with KAT transcripts detected at the highest level compared to the other TKP transcripts. Except a faint increase of KAT3 transcript, we observed no effect, or only marginally either increased or decreased levels of transcripts encoding the other KAT enzymes in response to IFN-γ exposure. Our observations are in line with the effects of IFN-γ observed in human fibroblasts [44] or neuronal cells [13], as there is no consensus in earlier studies regarding the response of the KAT enzymes to IFN-γ treatment. Whereas increases in the levels of KAT 1 and KAT 2 were observed in fetal astrocytes following IFN-γ treatment [14], no effect on the levels of transcripts encoding these enzymes was observed in neuronal cells [13]. In neuroblastoma cells, the IFN-γ treatment did not modify the levels of transcripts encoding KAT1 and KAT2 [13].

In the NAD branch of the TKP, KYN serves as a substrate for KMO, resulting in the production of 3-hydroxykynurenine (3HK) which is further degraded to 3-hydroxyanthranilic acid (3HAA) through the action of Kase. We found that rat pancreatic islets (but not INS-1 cells) constitutively express KMO under basal unstimulated condition. 48 h-exposure of rat islets to HGHF induced an upregulation of transcripts for KMO (7.6 fold; $p < 0.05$). IL-1β also upregulated the KMO transcripts (19 fold; $p < 0.01$) and KMO protein (2.5 fold; $p < 0.05$). By contrast, IFN-γ exposure (or H_2O_2) did not significantly affect KMO mRNA, under our experimental setting, and it did not potentiate the HGHF-induced KMO expression. Our immunohistochemical data suggest that HGHF activates accumulation of KMO protein mostly in rat β-cells as shown by our FACS-purified cell experiments. This is in accordance with the data related to KMO mRNA accumulation in basal or IFN-γ + HGHF conditions, in the β-cell fraction. In human skin fibroblast cultures, a lack of effect on transcripts encoding KMO following IFN-γ treatment [40] was also reported, while Heyes et al. [47] mentioned a small increase in KMO activity in IFN-γ exposed monocytes. Concerning IL-1β, it induced an up-regulation of KMO transcripts in human hippocampal progenitor cells [45].

Rat pancreatic islets (but not INS-1 cells) constitutively express Kase under basal unstimulated condition. FACS-purified cell experiments indicated that Kase transcripts were present in β-cells as in non β- islet cells. Levels of transcripts encoding Kase enzyme were not significantly modified in response to IFN-γ, IL-1β, HGHF, H_2O_2 or the combination HG/HF + IFN-γ.

A similar pattern of expression was observed for QPRT, with detectable QPRT transcripts in β-cells as in non β-cells in basal condition, no significant alteration in response to IFN-γ, IL-1β, H_2O_2 or the combination HGHF + IFN-γ, and a moderate but significant increase in response to HGHF.

Rat pancreatic islets and INS-1 cells did not express ACMSD either in basal unstimulated condition or in response to IFN-γ, IL-1β, HGHF, H_2O_2, or the combination HGHF + IFN-γ.

Rat pancreatic islets as well as INS-1 cells constitutively express NAMPT under basal unstimulated condition. FACS-purified cell experiments indicated that NAMPT transcripts were present in β-cells as in non β-cells. Levels of transcripts encoding NAMPT enzyme were upregulated in response to IFN-γ (1.6 fold $p < 0.01$), HGHF (2.4 fold; $p < 0.05$), IL-1β (2.3 fold; $p < 0.01$), while they remained unchanged after H_2O_2. At variance with lack of data related to expression and effect of TKP enzymes upstream NAMPT in islet cells, there are several reports indicating that NAMPT expression is detected in human islets (both in β- and non β-cells) and increased by high glucose [48,49]. This is in line with our present findings in rat islets. NAMPT is a rate-limiting enzyme in the mammalian NAD^+ salvage and it exists in two known forms, intracellular NAMPT (iNAMPT) and a secreted form, extracellular NAMPT (e NAMPT). eNAMPT is also known as both pre-B cell colony enhancing factor (PBEF) due to its function as a cytokine, and visfatin due to its role as an adipokine. It may also act as an extracellular enzyme converting extracellular nicotinamide to nicotinamide mononucleotide (NMN). NMN is an intermediate product in NAD^+ salvage pathway, may be taken up by cells and is utilized to generate NAD^+/NADH.

Finally, to summarize our present findings, despite the lack of IDO1, the normal rat islet constitutively expresses transcripts encoding the different enzymes of the KYNA branch of the TKP (KATs). It also express enzymes of the QUIN/NAD branch (KMO, Kase, QPRT), while the lack of ACMSD expression makes PIC or glutaryl-CoA production unfeasible. Our data also show that islet IDO1 and KMO are main targets regulated by environmental conditions associated to obesity/diabetes, since IDO1 expression is activated by cytokines (but not by HGHF or oxidative stress) while KMO expression is prominently activated by HGHF and IL-1β (but not or to lesser extent, by IFN-γ, oxidative stress or glucocorticoid). The many discrepancies identified here between islet cells and other cell types in response to cytokines or HGHF, reflect probably intrinsic differences across cell types in transcription of genes encoding TKP enzymes and in their abilities to form active TKP metabolites such as KYN, KYNA or QUIN [47]. The enzymes participating in the TKP, when expressed, are distributed at similar levels in both rat β-islet cells and non β-islet cells, except α-cells which harbor more KMO and QPRT. Also in the central nervous system, the TKP enzymes are found differently distributed among the different cells: the microglial cells harbor little KAT, and the astrocytes contain hardly any KMO with KYNA production therefore attributed mainly to astrocytes, while microglial cells are primarily responsible for the synthesis of QUIN [30].

The physiological role of the TKP in pancreatic islets is not known. Since the first description of the TKP in CNS thirty-some years ago, this pathway stays inclined towards unique properties as an interface between the immune system and neuronal signaling, since it is sensitive to inflammatory signaling and its products have neuromodulatory properties. Moreover, recent studies in yeast, worm, flies and mice have now established that TKP metabolism can also function as a regulator in lifespan and cancer [37]. In addition to its participation in NAD^+ homeostasis, the TKP generates a number of biologically active intermediate metabolites. QUIN is an excitatory (excitotoxic) agent at NMDA-type glutamate receptors and synergizes with 3-HK to produce oxidative stress. KYNA is an inhibitor of glutamate neurotransmission and possibly an antagonist at nicotinic α7 receptors. In addition to NMDA, AMPA and nicotinic α7 receptors, KYNA is reported to interact with GPR35 [50] and aryl hydrocarbon receptors [50].

Support for a tolerogenic role for TKP activation in islets in vivo has come from the observation that adenovirus-mediated IDO expression in transplanted NOD islets prolonged their survival in NOD/SCID recipients that received diabetogenic splenocytes [51,52]. These effects were in part attributed to local depletion and perturbation of the function of activated B and T lymphocytes by local depletion of free Trp [51,52]. Although Trp depletion is the favored mechanistic explanation for the tolerogenic effect of IDO1 activation, KYN and PIC can directly inhibit T-cell proliferation [51,52]. The release of Trp metabolites such as 3-HK, 3-HAA, QUIN and PIC could also provide bystander inhibition of immune cell function. Concerning the potential effects of the TKP on the insulin secretion, information are very scarce and poorly

informative, with a paper in the 70s which claims 3-HK and 3-HAA acutely block leucine-stimulated insulin secretion by rat islets [53], and to the best of our knowledge, no study related to effect of TKP metabolites on GSIS (which is the best reflection of the functional health of the beta-cell).Therefore our report that KYN significantly amplified GSIS by normal rat islets is the first one. Of course, it remains in the near future to identify the mechanisms by which KYN or KYNA potentiate GSIS. This will not be an easy task since sources of KYN or KYNA may act intracellular as well as extracellular, separately or additively, to modulate GSIS. The acute effect of KYN is possibly related to its ability (or its metabolites) to interact with some specific receptors. Our future studies should include the expression of receptors for KYN and its metabolites, such as AHR, GPR35, NMDA and AMPA proteins in normal rat islets.

In conclusion, we have demonstrated for the first time in normal rat pancreatic islets, 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 is 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. Therefore the pancreatic islets may represent a new target tissue for inflammation and glucolipotoxicity to activate the TKP pathway. Of course the next step will be to evaluate the functional impact the various TKP metabolites have upon the islet cells. Most importantly, since inflammation is now recognized as a crucial mechanism in the development of the metabolic syndrome [46] and more specifically at the islet level [34], it is therefore 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.

Authors' contributions

JJL participated in the design of the study, performed islet isolation, islet and cell culture, carried out RNA and western blot analyses and immunohistochemistry, performed all statistical analyses, analyzed and interpret the data and wrote the manuscript; CC carried out the TKP metabolite analyses; BG carried out the islet cell sorting; MK and VA participated in the design and coordination of the study; SR, DB and JM participated in the design of the study, performed the islet perifusion experiments, acquired and analyzed the data; BP conceived the study, participated in its coordination, obtained funding for the study and drafted the manuscript. All authors helped to revise the first draft of the manuscript and all authors approved the final manuscript. BP is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Duality of interest

BP received a research grant from Metabrain Research. No other potential conflicts of interest relevant to this article were reported.

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