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TSPO in pancreatic beta cells and its possible involvement in type 2 diabetes

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ABSTRACT

Amyloidosis forms a large family of pathologies associated with amyloid deposit generated by the formation of amyloid fibrils or plaques. The amyloidogenic proteins and peptides involved in these processes are targeted against almost all organs. In brain they are associated with neurodegenerative disease, and the Translocator Protein (TSPO), overexpressed in these inflammatory conditions, is one of the target for the diagnostic. Moreover, TSPO ligands have been described as promising therapeutic drugs for neurodegenerative diseases. Type 2 diabetes, another amyloidosis, is due to a beta cell mass decrease that has been linked to hIAPP (human islet amyloid polypeptide) fibril formation, leading to the reduction of insulin production. In the present study, in a first approach, we link overexpression of TSPO and inflammation in potentially prediabetic patients. In a second approach, we observed that TSPO deficient rats have higher level of insulin secretion in basal conditions and more IAPP fibrils formation compared with wild type animals. In a third approach, we show that diabetogenic conditions also increase TSPO overexpression and IAPP fibril formation in rat beta pancreatic cell line (INS-1E). These data open the way for further studies in the field of type 2 diabetes treatment or prevention.

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1. Introduction

The origin and the key factors of the development of several amyloid diseases resulting in various human pathologies remain unknown [1]. Amyloidosis share the common characteristics of amyloid deposit, known as amyloid fibrils or plaques, originating

from globular proteins. Although many studies have permitted to describe, *in vitro*, how may occur the transformation of monomeric globular protein into insoluble fibrillar proteins, the process occurring *in vivo* is not yet clearly explained [1]. Amyloidogenic proteins and peptides are synthesized in almost all organs, some of them are plasma circulating proteins thus targeting several organs, some others remain in the tissue where they are synthesized [2]. Amyloid fibrils located in the brain have been the subject of intense studies due to their pivotal participation in neurodegenerative pathologies, such as Alzheimer's, Parkinson's and Creutzfeldt-Jakob's diseases [3–5]. Similarly, amyloid fibrils located in the pancreas (composed mainly of hIAPP, human Islet Amyloid Polypeptide) have also been largely studied because of their implication in type 2 diabetes (T2D) [2]. It has long been described that inflammation participates in the pathogenesis of T2D [6] as well as in neurodegenerative diseases [7] and involves oxidative stress processes [8,9] (Fig. 1). The regulation of the inflammatory pathway and of reactive oxygen species (ROS) production have become

Abbreviations: BMI, Body Mass Index; CTR, Calcitonin Receptor; GLUT2, Glucose Transporter 2; IAPP, Islet Amyloid Polypeptide; IL-1beta, Interleukin-1 beta; MRI, Magnetic Resonance Imaging; PET, Positron Emission Tomography; PDX1, Pancreatic and Duodenal homeobox 1; RAMP, Receptor Activity-Modifying Protein; ROS, Reactive Oxygen Species; SOD, SuperOxide Dismutase; ThT, Thioflavin T; TNF alpha, Tumor Necrosis Factor alpha; TSPO, 18 kDa Translocator protein.

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targets for new therapies [10,11]. Among the signalling pathways and transcriptional factors involved in the inflammation, finding the right therapeutic target is the most important and difficult approach for drug design [12]. Targeting simultaneously several inflammatory pathways has been proposed to be better than focusing on only one [11]. Conversely to previous therapeutic strategies enhancing ROS production to kill cancer cells, it has been proposed to use antioxidant repurposed drugs to clear ROS production [13]. A recent review [10] has analysed the various mechanisms of antioxidant production in response to stress and described the potential effect of small molecules in an upcoming field of therapy in neurodegenerative disorders.

Among the various key sites and factors for the development of the fibrillation process, plasma membrane and mitochondria have largely been described [1,14,15]. The latter is essential for cell function, and its dysfunction has been proposed to generate ROS that increase fibril formation from amyloid proteins [16]. Among the proteins involved in ROS down-regulation in mitochondria, the Translocator Protein (TSPO), an 18 kDa protein located mostly in the outer membrane of mitochondria, has been largely described [17,18] (Fig. 1). On one hand, TSPO ligands have been described to reduce inflammasome activation through reduction of ROS production [19]. On the other hand, TSPO deficiency resulted in enhanced activation of inflammasome pathway promoting more proinflammatory cytokine production [20]. These results demonstrate that TSPO is a crucial modulator of the inflammation process. Moreover, TSPO has been exponentially used over the last decades as a neuroimaging biomarker of brain diseases linked to inflammation [21] due to its overexpression in those pathologies [22].

Amyloidosis diseases have been initially characterized post-mortem with the detection of amyloid deposits in tissue sections [23]. Over last decades, new detection techniques have been developed like positron emission tomography (PET) with radiotracer targeting different proteins among which TSPO using a large family of ligands [24]. Colocalization of TSPO and amyloid fibrils has been described in animal model by mixing the PET and monoclonal antibodies techniques [25]. Recently, TSPO quantification in patient with Alzheimer's disease has been carried out with PET radiotracer [26]. Several tracers have been developed to target directly the proteinopathies with positron emission tomography (PET) [27] and magnetic resonance imaging (MRI) has also been recently used with fluorinated probes [28] or nanotheranostics platform [29]. An improved resolution (from mm to μm) has been obtained in a mouse model using combined PET/MRI approaches [30]. Type 2 diabetes mellitus in human has been studied through detection of amylin aggregates using PET imaging probes [31], but no study of

inflammation using TSPO PET tracer is available for this pathology in human, whereas data are available for pancreatic cancer in mouse models [32].

Increasing evidence has placed inflammation at the centre of the pathogenesis of neurodegenerative diseases linked with amyloid protein aggregation [7]. However, it is not fully understood what triggers this phenomenon. It is interesting to underline that inflammation and oxidative stress are also markers of prediabetic and diabetic states [33] and that mitochondrial oxidative phosphorylation is impaired by amyloid proteins [16], suggesting an implication of mitochondria in the process. However, no study has been performed to link regulation of inflammation, ROS production by TSPO and hIAPP fibril formation in type 2 diabetes.

TSPO ligands have been found to reduce neuroinflammation, ROS production and amyloid fibril formation *in vitro* [34,35] and *in vivo* [36–38] (Fig. 1). Although the mechanism of neuroprotection is not clear, it has been reported that the TSPO ligand Ro5-4864 promotes production of anti-inflammatory cytokines [39] and increase microglia phagocytotic ability [36]. The implication of TSPO in proliferation/cell death has been questioned and it has been concluded that amyloid-induced death seems to be independent of TSPO [40]. However, the variety of ligands available to target TSPO [41] that have different chemical structures and show neuroprotection reducing the accumulation of β -amyloid peptide [42,43] or tau [36], two proteins involved in Alzheimer's disease and tauopathies, may be questioned. Extending this kind of study to type 2 diabetes would be beneficial for therapeutic approaches and for a better mechanism description.

TSPO deficiency in mice shows significantly higher levels of amyloid proteins and plaques as well as increased level of proinflammatory cytokines in the brain [44], making TSPO a potential therapeutic target in line with previous studies [41]. In the intestine, global TSPO knockout in mice has been shown to exacerbate ulcerative colitis pathology with an extensive inflammation [45,46] in agreement with previous study making TSPO a therapeutic target [17,47]. Moreover, TSPO conditional knock-out mice exhibit prediabetes symptoms [48].

The presence of TSPO in pancreas has been characterized many years ago [49] using classical radioactive ligands, but with some difference between animals. Histological approach has revealed the presence of TSPO in human pancreas [50]. More recently, the localisation of TSPO has been performed on healthy human volunteers using TSPO specific ligands for PET scanning [51–53]. An increased pancreatic expression of TSPO has been characterized in obese (*fa/fa*) rodents [54]. However, no data are available for TSPO expression in diabetic patients.

In the present work, we evaluate the presence of TSPO in overweighted patients, potentially prediabetic, and its link with inflammation. We also estimate the same parameters in the rat beta pancreatic cell line (INS-1E) upon culture in diabetogenic conditions. We further study the kinetics of hIAPP fibrillation on these cells. Finally, we determined the pancreatic beta cell mass, the insulin secretion and the kinetics of hIAPP fibril formation in TSPO deficient rat. These data suggest that TSPO may constitute a diagnostic and therapeutic target for type 2 diabetes and opens the way to further studies.

2. Material and methods

2.1. Human islets

Human islets batches (17 donors) were provided by the Cell Therapy Unit (Saint-Louis Hospital, Paris) among which 8 and 9 donors with a body mass index (BMI) < 25 and a BMI > 25, respectively. Total RNAs were isolated (RNeasy Mini Plus Kit; QIAGEN,

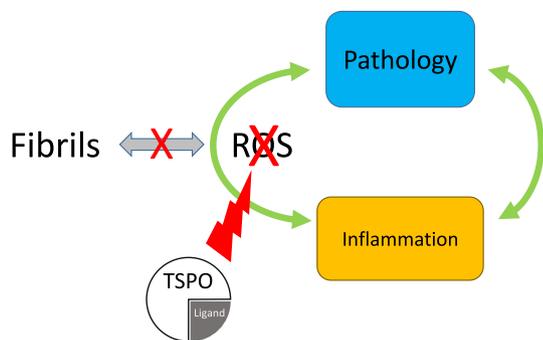


Fig. 1. Schematic representation showing the relationship between pathology, inflammation, fibril formation and the potential regulation by TSPO. Amyloidosis pathologies are linked to inflammation producing ROS that favours fibrils formation involved in the death of cells. TSPO ligands have been described to reduce inflammation, ROS production and thus fibril formation, acting as a protecting agent.

Hilden, Germany) and reverse transcribed into cDNA with SuperScript transcriptase (Applied Biosystems, Foster City, CA, USA). Gene expression was quantified by real-time PCR using SYBR Green Supermix (Applied Biosystems) in a QuantStudio1 thermal cycler (ThermoFisher Scientific, Montigny-le-Bretonneux, France). The Ct value obtained for each specific gene product was normalized for 18 S rRNA and expressed as the fold change of the value in the control condition. Primer sequences are available upon request.

2.2. Animals

Sprague-Dawley wild-type (WT) or TSPO-KO (SD-Tspo emVp1) male rats generated by Vassilios Papadopoulos (University of Southern California) [55] and donated to Didier Morin (IMRB, INSERM U955) were used to obtain pancreas for the studies reported in this manuscript.

2.3. Islet isolation and glucose-stimulated insulin secretion

Pancreas from rats were digested with liberase (Roche, Boulogne-Billancourt, France) in Hank's balanced salt solution (HBSS, 137 mM NaCl, 5.36 mM KCl, 4.17 mM NaHCO₃, 0.88 mM MgSO₄, 0.44 mM KH₂PO₄, 0.34 mM Na₂HPO₄, 1.27 mM CaCl₂, 10 mM HEPES, and 0.5 % bovine serum albumin [BSA]) and islets of Langerhans purified from exocrine tissue by discontinuous density-gradient centrifugation (Histopaque 1077; Sigma-Aldrich, Saint-Quentin-Fallavier, France) [56]. The islets were hand-picked under a binocular microscope (Leica Microsystems, Wetzlar, Germany) and used for hIAPP fibrillation or glucose-stimulated secretion experiments, or frozen until used. For glucose-stimulated secretion experiment, freshly isolated islets were cultured and stimulated as previously described [57].

2.4. Immunohistochemistry, immunofluorescence, and morphometry

Pancreas were fixed in 3.7 % formalin solution, embedded in paraffin, and cut into 5-mm thick sections. Morphometrical parameters (beta-cell fraction, islet size, and density) were evaluated from 4 sections per pancreas after immune histochemistry using a mouse polyclonal anti-insulin (Sigma-Aldrich), and a secondary antibody coupled to horseradish peroxidase (Jackson ImmunoResearch, Westgrove, PA, USA). Enzyme substrate was DAB+ (Dako-Agilent, Les Ulis, France). Morphometrical parameters were determined as previously described [58]. Pictures were taken on a slide scanner (Hamamatsu slide scanner 2.0 HT, Hamamatsu, Massy, France) and the stained areas were measured using the software Calopix (Tribun Healthcare, Paris, France).

2.5. INS-1E cells culture

Rat insulinoma-1 (INS-1E) pancreatic beta-cells were grown in culture medium containing RPMI 1640 supplemented with penicillin (100 units/ml), streptomycin (100 mg/ml), pyruvate (1 mM) (all from Invitrogen, Paris, France), beta-mercapto-ethanol (50 mM) (Sigma-Aldrich), and 10 % heat-inactivated calf serum (Dutscher, Bernolsheim, France). The cultures were maintained at 37 °C in an incubator humidified 95 % air, 5 % CO₂. INS-1E cells were plated at a density of 300 000 cells/well in a 24-well plate. Following 24 h of culture, cells were treated for another 24 h-period with BSA alone or complexed with palmitate (Bertin Technologies, St Quentin en Yvelines, France). The molar ratio of palmitate to BSA was 5:1. The fatty acid stock solution was diluted in RPMI-1640 Medium (Invitrogen, Paris, France) supplemented with 1 % (vol/vol) FBS to obtain the required final concentration. At the end of the culture period,

total RNAs were extracted, following manufacturer's recommendation (Macherey-Nagel, Hoerd, France). Reverse transcription as well as gene expression analysis were performed as described in the human islets section.

2.6. hIAPP fibrillation

INS-1E cells and rat islets were plated respectively at a density of 30 000 cells/well or 50 islets/well in a 96-wells black plate (flat bottom, Greiner Bio-One, Courtaboeuf, France). Following 24 h of incubation, the medium was replaced with 100 µl of fresh medium containing 50 µM of indicated peptide as previously described [59]. 10 µl (final concentration 20 mM) of Thioflavine T (ThT) was added in each well in order to monitor fibril formation. The fluorescence was measured at 30 °C from the top of the plate every 30 min with excitation filter 440 nm and emission filter 485 nm for a 15 h (INS-1E cells) or a 30 h period (rat islets) using a spectrophotometer (TECAN, Lyon, France). For rat islets, the plate was taken out of the incubator to measure the ThT fluorescence each 30 min during 30 h. For INS-1E cells, ThT fluorescence was measured continuously for 15 h, with acquisition point taken every 10 min.

2.7. Statistics

Each experiment was performed at least 3 times in triplicates. Results are expressed as means ± SEM. Statistical significance was determined using Student's test (*, p < 0,05; **, p < 0.01; ***, p < 0.001).

3. Results

3.1. TSPO in human islets from pancreas of healthy and prediabetic post-mortem donors

In order to determine parameters acting on TSPO expression, we compared mRNA levels of TSPO and proteins known to be implicated in diabetes (insulin, glucose transporter, inflammatory proteins, ROS regulating proteins, hIAPP and component of its receptors AMY 1–3 [60]) in pancreatic islets of patients with BMI below (<25, 7 donors) and above overweight (>25, 9 donors), the latter being described as a risk factor for pancreatic diseases [61,62]. Our data show (Fig. 2A) that TSPO mRNA expression is significantly increased in patient with a BMI over 25 compared to control patient with a BMI below 25. Moreover, Interleukin-1 beta (IL-1beta) mRNA, a proinflammatory cytokine known to induce glucotoxicity [63], and superoxide dismutase 2 (SOD2) mRNA, a mitochondrial protein that down regulates the level of ROS [64], are also significantly overexpressed in patient with a BMI over 25 (Fig. 2B). It has to be mentioned that mRNA expression of SOD2 level has been previously analysed as reflecting the protein expression and enzyme activity in rat pancreatic islets [64]. Conversely, mRNA level of Insulin is decreased in patient with a BMI over 25 (Fig. 2A) in agreement with the risk factor for pancreatic diseases. Finally, mRNA of SLC2A2 coding GLUT2, a glucose transporter, tumor necrosis factor alpha (TNF alpha), SOD1, hIAPP, RAMP1,2,3 (receptor activity modifying protein) and calcitonin receptor (CTR, belonging to the GPCR family), forming the hIAPP receptors AMY 1–3 [60] are not modified (Fig. 2B and C). The difference observed between IL-1 beta and TNF alpha mRNA regulation might suggest different tissue specific immunoenhancing effects [65,66] that have to be further studied. The non-significant expression modification of hIAPP and component of its receptor might suggest that they are not involved yet at this prediabetic stage. Our results suggest a positive correlation between TSPO, inflammation and ROS production in prediabetic conditions. An extrapolation to diabetes has been suggested

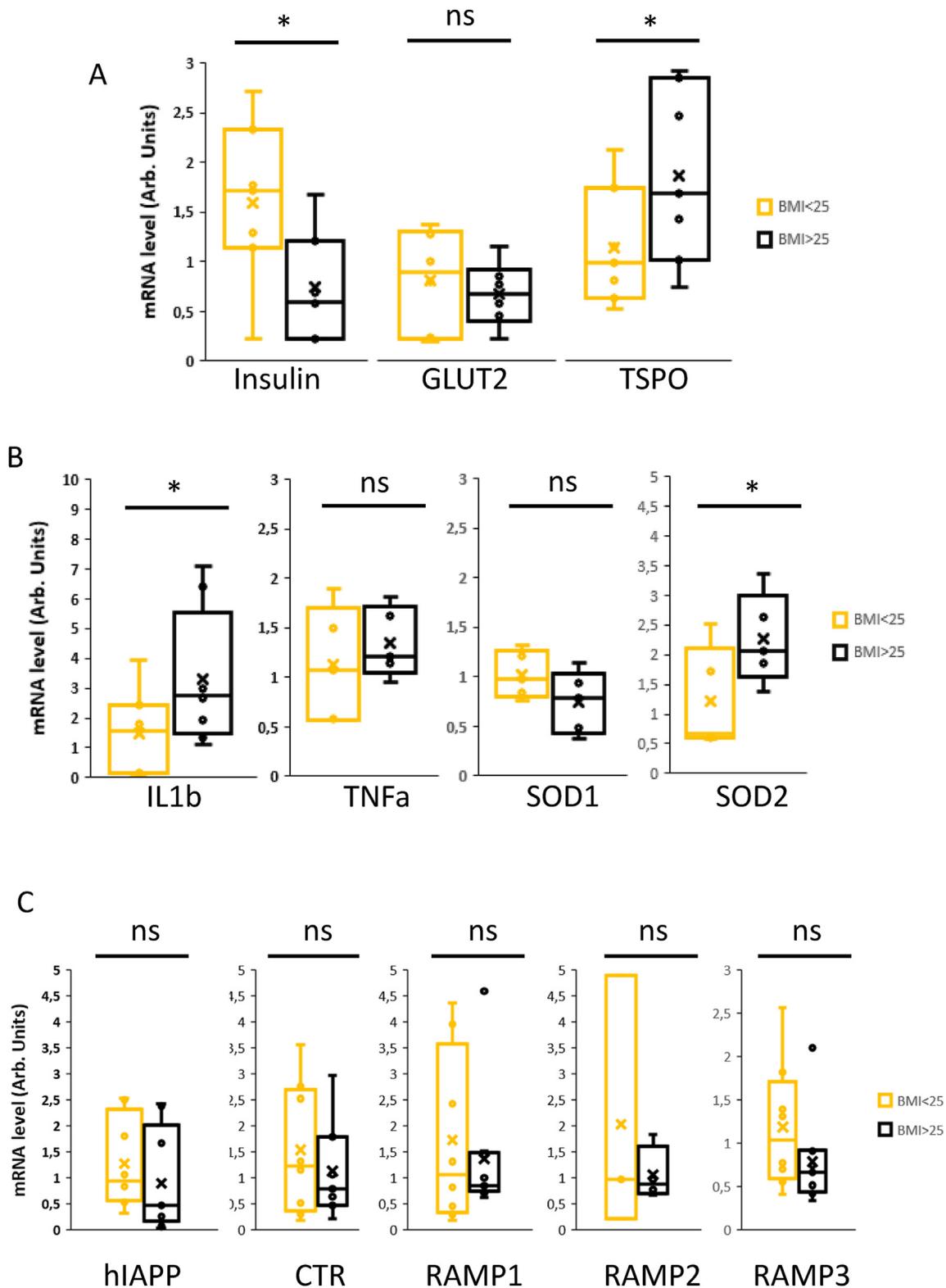


Fig. 2. Comparison of mRNA level in human islet from patients with BMI below (orange) and above 25 (black). mRNA level of (A) Insulin, GLUT2 and TSPO, (B) inflammatory cytokine (IL-1 β and TNF- α) and SOD1 and 2, (C) CLTR, hIAPP, RAMP 1,2,3. *, $p < 0.05$; ns, not significant.

in a previous study indicating that human pancreatic islets (10 donors) exposed 72 h to a cytotoxic cytokine combination of IL-1 beta, TNF alpha and interferon gamma (IF- γ), show an upregulation of TSPO (protein and mRNA) and an increase in beta-cell death

associated with apoptosis and mitochondrial swelling [67].

3.2. TSPO in pancreas from wild type and knock-out rat

To further test the role of TSPO in pancreas, we analysed the morphology of pancreatic islet of wild type (WT) and constitutive global TSPO knock-out (TSPO-KO) Sprague-Dawley rats [49]. The global beta cell content as well as the islet average size were not modified, while the islet density was significantly decreased in TSPO-KO rat pancreas (Fig. 3).

We compared insulin secretion by WT and TSPO-KO pancreas in diabetogenic conditions. Pancreatic islets were cultured and probed for insulin secretion stimulated 1 h by low glucose, then 1 h by high glucose and finally 1 h with KCl, this later condition allowing insulin secretion in the absence of glucose metabolism. Low glucose (2.5 mM) has no effect on WT islets whereas it significantly stimulates insulin secretion in the TSPO-KO islets (Fig. 4A). Conversely, high glucose (16.7 mM) and KCl (50 mM) strongly stimulate insulin secretion in WT, whereas both conditions have a significantly reduced effect on TSPO-KO (Fig. 4B and C). These data reveal a pancreatic beta cells dysfunction in TSPO-KO rats since insulin secretion is not correlated to glucose concentration. However, this dysfunction is not observed *in vivo* in rats. Indeed, when glucose is injected (1 g/kg, Fig. S1, panel A) there is no differences between WT and TSPO-KO rats. Moreover, glucose and insulin tolerance in WT and TSPO-KO rats (Fig. S1, panels A and B) do not show any significant differences [68,69]. These experiments revealed a difference between animals (*in vivo*) and islets (*in vitro*), probably due to different regulation other than glucose metabolism. In absence of TSPO, insulin secretion by beta cells is dysregulated and not linked to glucose concentration. This dysfunction of pancreatic beta cells has been previously described in *Tspo* cKO mice that exhibited sustained hyperglycemia whereas TSPO-KO rat do not [48].

We also compare the IAPP fibrillation on WT and TSPO-KO pancreatic islets. Since IAPP from rat (rIAPP) have mutations that prevent its fibrillation, we used human IAPP (hIAPP) to analyse if TSPO deletion has an effect. We thus performed hIAPP fibrillation by adding 50 μ M of synthetic peptide to pancreatic islet issued from WT and TSPO-KO (Fig. 5). The absence of TSPO slows down the kinetics of fibrils formation followed by ThT fluorescence (half-time increases from ~1 h to ~5 h for WT and TSPO-KO, respectively), but seems to increase the amount of fibrils as suggested by the higher level of fluorescence. In TSPO-KO rats the basic level of insulin is increased compared to WT (Fig. 4A) and it has been previously described that insulin prevents the hIAPP fibrillation [70], this might explain why the fibrillation is delayed, on one hand. However, on the other hand, the absence of TSPO prevents the inhibition of ROS production that increases the fibrils formation as soon as the small oligomers are formed, this might explain why the final

amount of fibrils is increased.

3.3. TSPO in rat insulinoma cell line (INS-1E) in prediabetic and diabetic conditions

We used rat insulinoma cell line (INS-1E) to decipher at a cellular level the links between TSPO, inflammation, ROS in prediabetic and diabetogenic conditions and IAPP fibrillation. We performed INS-1E cell cultures (24 h) in the presence of low (5 mM) glucose concentration in the absence and in the presence of palmitate (0.4 %), and high (30 mM) glucose concentration in the presence of palmitate (0.4 %) (Fig. 6). At low glucose plus palmitate (G5+Palm), conditions similar to prediabetic donors, we observed a negative relation between expression of mRNA levels of TSPO and insulin (Fig. 6A), with significant increase in TSPO and decrease in insulin, similarly to prediabetic donors (Fig. 2A). However, no significant differences were observed for the mRNA levels of GLUT2 and pancreatic and duodenal homeobox 1 (PDX1), a transcription factor known to stimulate insulin expression (Fig. 6A) as also observed for prediabetic donors. We noticed a slight increase in the proinflammatory cytokine TNF alpha, but not in SOD1 and SOD2 which are regulated by ROS (Fig. 6B), conversely to prediabetic donors where only SOD2 is increased (Fig. 2B). In these prediabetic conditions (G5+Palm), the kinetics of hIAPP fibrillation do not show significant differences compared to low glucose alone (Fig. 6C). We believe that TSPO may somehow protects cells in prediabetic conditions, but further studies with TSPO-KO cells are needed to confirm our hypothesis. At high glucose plus palmitate, diabetogenic conditions (G30+Palm), we observed a significant decrease in insulin mRNA compared to prediabetic conditions (Fig. 6A), as well as significant decrease in GLUT2 and PDX1 mRNA compared to glucose alone (Fig. 6A). At high glucose plus palmitate, TNF alpha, SOD1 and SOD2 mRNA levels are similar to the levels observed in the presence of glucose alone (Fig. 6B). In these diabetic conditions, the kinetics of hIAPP fibrillation are very different compared to prediabetic, the maximum ThT fluorescence is increased suggesting a higher amount of fibrils (Fig. 6C). At high glucose plus palmitate, rIAPP mRNA is significantly reduced (Fig. 6D) in agreement with insulin decrease. Whatever the prediabetic or diabetic conditions, no significant modification of CLTR and RAMP1,2,3 mRNA levels were observed (Fig. 6D). In diabetic conditions (G30+Palm), apoptosis is strongly activated [71] leading to down regulation of many proteins that may explain why TSPO is not anymore overexpressed, thus unable to protect cells against cell death and hIAPP fibrils formation. We hypothesis that TSPO overexpression might have a protective effect on cell survival in diabetic conditions, further studies are needed to confirm our hypothesis.

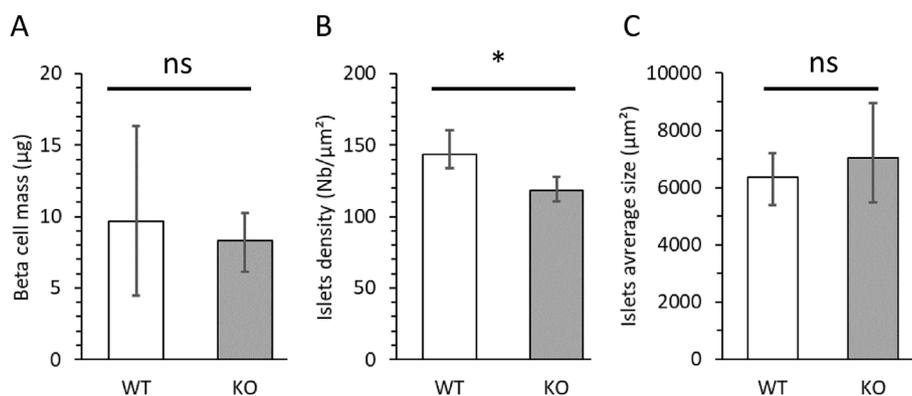


Fig. 3. Rat pancreatic islet morphology for WT and TSPO-KO animals. (A) Beta cell mass, (B) Islets density, (C) Islets average size. *, $p < 0.05$; ns, not significant.

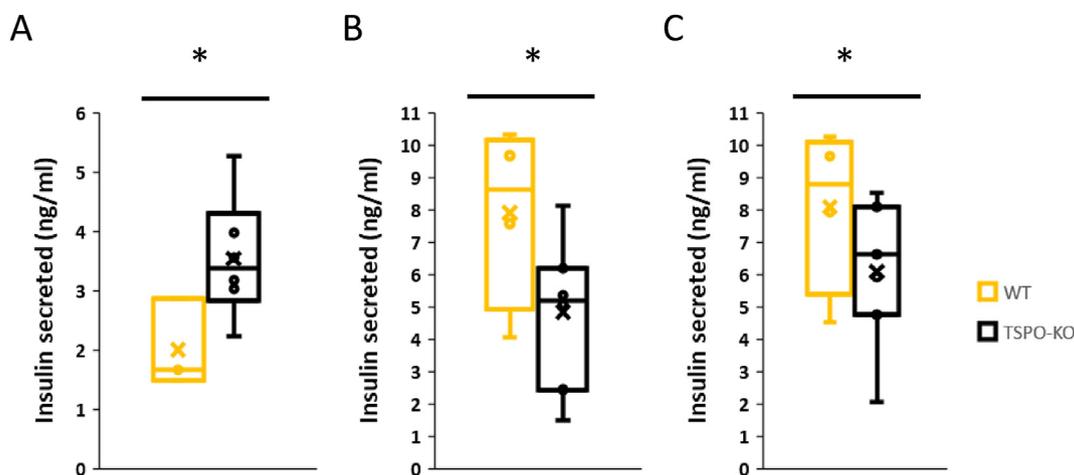


Fig. 4. Insulin secretion by cultured pancreatic rat islets from WT and TSPO-KO animals stimulated by glucose (2.5 and 16.7 mM, panels A and B, respectively) or KCl (50 mM, panel C). 11 islets from pancreas of WT and TSPO-KO rats (2 and 4 animals, respectively) were cultured and insulin secretion was measured in duplicate. *, $p < 0.05$.

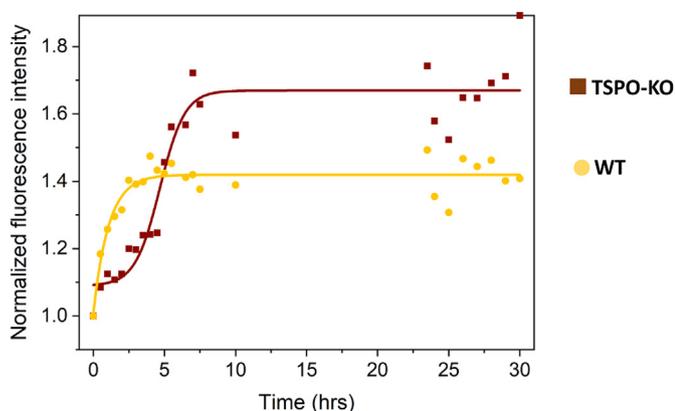


Fig. 5. Kinetic of hIAPP fibrillation on pancreatic rat islets. Fibrillation of hIAPP (50 μ M) followed using the ThT (20 mM) fluorescence is shown for pancreatic islets issued from WT and TSPO-KO rats in yellow and dark red, respectively. Excitation and emission wavelength, are 440 and 485 nm, respectively.

4. Discussion and perspectives

In human pancreatic islets, an upregulation of TSPO has been previously described in response to damage associated with inflammation leading to cell death [67], a critical step towards type 2 diabetes since insulin is not anymore secreted in sufficient amounts. However, no data are available upon a link between TSPO and prediabetic conditions.

Our data demonstrate, in human pancreatic islets from patients with low and high BMI, a link between prediabetic conditions and overexpression of TSPO mRNA coupled to insulin mRNA decrease. This may suggest a regulatory function of TSPO in agreement with data gained in TSPO-KO mice [48]. Indeed, these mice show hyperglycemia, a sign of prediabetes and it was proposed that it was induced by an increase in glucose production [48]. However, no significant difference in basal glucose concentration was observed between WT and TSPO-KO rats [68,69] that could be due to species differences. In TSPO-KO rat pancreatic islets, we show that basal insulin secretion is higher than in WT rats. These data suggest a beta cells dysfunction in TSPO-KO animals. Moreover, in WT rat pancreatic islets, we show that high concentrations of glucose or KCl, simulating diabetic conditions, induce a fourfold increase in insulin secretion. However, similar treatment for TSPO-KO rat

pancreatic islets only induces a small increase in insulin secretion confirming beta cells dysfunction in rat TSPO-KO. Our data show that, while the islet mass is not modified in the TSPO-KO pancreas, the density is diminished suggesting a reduction of small islets known to be the most insulin secretive [72]. This should have led to a reduced insulin secretion. However, the increased basal insulin level and the reduced stimulation by glucose or KCl demonstrate a dysfunction of the pancreatic islets. The implication of TSPO in the development of the islets and their function needs to be further investigated. When performing prediabetic treatment to rat insulinoma cell line (INS-1E), we observe a decrease in insulin mRNA expression and an increase in TSPO mRNA expression as observed in patient with high BMI. It has to be notice that TSPO is overexpressed only in prediabetic conditions that are not toxic to INS-1E cells, conversely to diabetic conditions that have been described as toxic [73]. We propose that this TSPO induction could act as a pancreatic beta cell protection as observed in the P19 cell line by regulating ROS production [74]. TSPO deficiency exacerbates inflammasome activation pathway [20] characterized to be linked to mitochondrial ROS [75]. The overexpression of TSPO is correlated with overexpression of inflammatory cytokine IL1-beta which has been previously described as linked to mitochondrial swelling and cell death [67]. There is a crosstalk between mitochondrial swelling and ROS production [76], the latter being described as inducing and stabilizing fibrils [77]. Mitochondrial dysfunction has been described in many neurodegenerative diseases [8] and its regulation a target to prevent them [22]. We did not observe a change in the kinetics of hIAPP fibrils formation between control and prediabetic conditions for INS-1E cells while TSPO is overexpressed. This may suggest a protective effect of TSPO in prediabetic conditions. However, in diabetic conditions, a significant increase in hIAPP fibrils formation has been observed, whereas TSPO has been reduced. Similarly, TSPO-KO pancreatic islets show an increase in hIAPP fibrils formation. Further studies are needed to measure ROS levels in these conditions. A previous study with mice lacking TSPO has shown significant higher level of beta amyloid plaques and more proinflammatory cytokines in brain [44] and suggest that TSPO has protective functions. Moreover, it has been described that TSPO ligands present neuroprotective effects by regulating mitochondrial functions [36,41,43,78,79]. No data are available upon pancreas on the use of ligand to image or to protect against hIAPP fibrils formation. However, TSPO-PET ligand have been used to localize pancreatic ductal carcinoma tumors in a mice model [80] as well as premalignant and pancreatic cancer lesions in human [81].

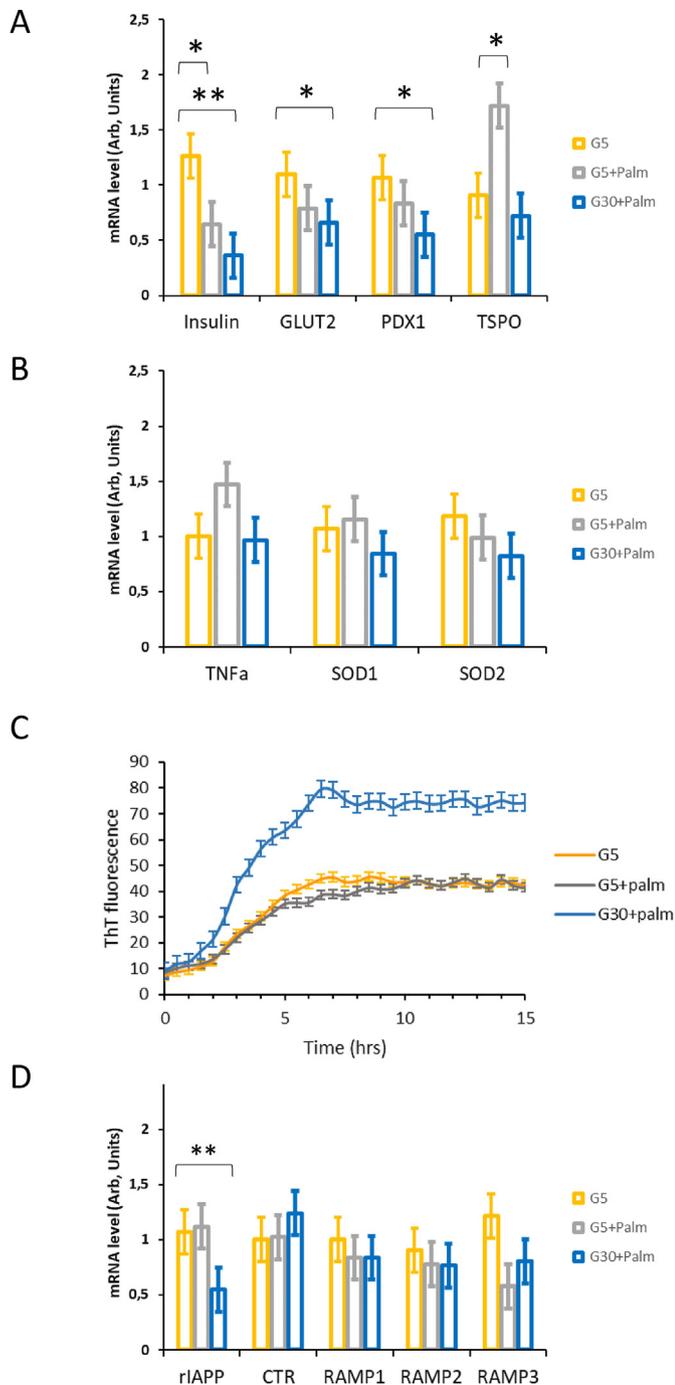


Fig. 6. Comparison of INS-1E cells cultured in normal (Glucose 5 mM, orange), pre-diabetic (Glucose 5 mM plus palmitate, grey) and diabetogenic (Glucose 30 mM plus palmitate, blue) conditions. (A) mRNA level of Insulin, GLUT2, PDX1 and TSPO, (B) mRNA level of TNF alpha, and SOD1 and 2, (C) ThT fluorescence to follow the kinetic of hIAPP fibrillation. (D) mRNA level of rIAPP, CLTR, RAMP1,2,3 independent experiments in triplicate for each condition. *, $p < 0,05$; **, $p < 0,01$; not significant if not indicated.

Further studies are needed to test the effect of TSPO ligands upon hIAPP fibril formation (Fig. 7) taking into account that the high affinity drug ligand PK 11195 has been described to induce a reduction of insulin secretion both in rats isolated pancreas perfused with a slightly stimulating glucose concentration [82], but also in human pancreatic islet in similar glucose conditions [83]. PK 11195 has also been characterized to reduce inflammasome

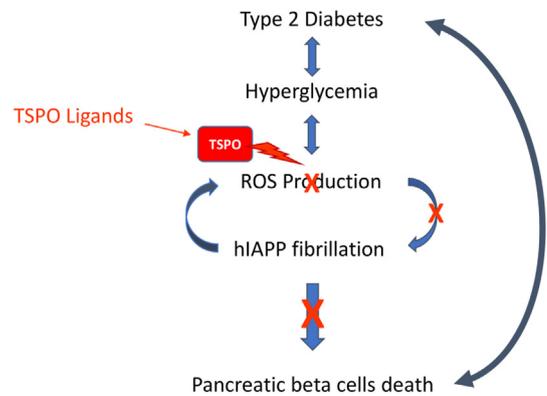


Fig. 7. Schematic representation of TSPO implication in hIAPP fibril formation involved in type 2 diabetes. Type 2 diabetes leads to hyperglycaemia partly due to death of beta pancreatic cells that produce insulin and regulate glucose level. This death process is governed by ROS generated and amplified by hIAPP fibrils formation. TSPO and its ligands are known as ROS regulators and thus a good candidate to reduce hIAPP fibrils formation and to preserve functional pancreatic beta cell mass.

activation and cytokine release [19]. This has to be linked to a previous study showing that reduction of inflammasome expression improves insulin-sensitivity in obese type 2 diabetic patients [84]. To better understand the role of TSPO, further studies with INS-1E cell where TSPO expression will be modified (over-expression or no expression), are also needed.

As a conclusion, TSPO could be used as a target to characterize the diabetic risk for patients or to analyse pancreas previous to transplantation. Indeed, an increase in TSPO protein can be detected using numerous radiotracers for TSPO positron emission tomography already available for imaging [8,85,86] and used in diverse human CNS pathologies [21].

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CRediT authorship contribution statement

Ghislaine Guillemain: Writing – review & editing, Visualization, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Lucie Khemtemourian:** Writing – original draft, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Juliette Brehat:** Methodology, Investigation, Formal analysis, Data curation. **Didier Morin:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Jamileh Movassat:** Resources. **Cécile Tourel-Cuzin:** Resources. **Jean-Jacques Lacapere:** Writing – review & editing, Supervision, Project administration, Methodology, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

No conflict of interest to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.biochi.2024.06.007>.

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