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# **The mitochondrial Translocator protein (TSPO): a key multifunctional molecule in the nervous system**

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## Abstract

Translocator protein (TSPO, 18 kDa), formerly known as peripheral benzodiazepine receptor, is an evolutionary well-conserved protein located on the outer mitochondrial membrane. TSPO is involved in a variety of fundamental physiological functions and cellular processes. Its expression levels are regulated under many pathological conditions, therefore TSPO has been proposed as a tool for diagnostic imaging and an attractive therapeutic drug target in the nervous system. Several synthetic TSPO ligands have thus been explored as agonists and antagonists for innovative treatments as neuroprotective and regenerative agents. In this review, we provide state-of-the-art knowledge of TSPO functions in the brain and peripheral nervous system. Particular emphasis is placed on its contribution to important physiological functions such as mitochondrial homeostasis, energy metabolism and steroidogenesis. We also report how it is involved in neuroinflammation, brain injury and diseases of the nervous system.

**Key words:** Translocator protein (TSPO), steroidogenesis, neuroinflammation, biomarker, neuropathology, neuroprotection

## Introduction

Discovered in 1977 as an alternative binding site for the benzodiazepine diazepam, the 18-kDa translocator protein (TSPO1) belongs to a family of tryptophan-rich sensory proteins (1). TSPO1 possesses a highly conserved sequence from plants to humans. It is widely present in most Eukarya, Archae, and Bacteria (2). *Tspo1* gene is located on the long arm of human chromosome 22 (q13.2) and encodes a hydrophobic 18kDa TSPO1 protein carrying 169 amino-acids (3, 4). Notably, TSPO from *Rhodobacter sphaeroides* (RsTSPO) shares only 34% sequence identity with the human and rat proteins while highly conserving functional properties (5).

TSPO is a ubiquitous mitochondrial protein located on the outer mitochondrial membrane (OMM) as part of a multimeric (140-200 kDa) protein complex (6) (7). TSPO consists of five hydrophobic transmembrane  $\alpha$  helices which are long enough to stretch over the bilayer membrane, with the carboxyl-terminus in the cytosol and a short amino-terminus in the intermembrane space of mitochondrion. Also, TSPO interacts with the so-called mitochondrial permeability transition pore (mPTP), formed by the 30 kDa voltage-dependent anion channel (VDAC) located in the outer mitochondrial membrane, and the 32 kDa adenine nucleotide translocator (ANT) in the inner mitochondrial membrane (IMM) (8). Indeed, VDAC allows the passage of nucleotides, metabolites and ions including calcium ( $\text{Ca}^{2+}$ ) between the mitochondrial inter-membrane space and cytoplasm (9). ANT exchanges free ATP with free ADP across the inner mitochondrial membrane (10). TSPO is involved in adjusting the opening and the closure of the mPTP. This could lead to either apoptosis via mPTP opening, or promotion of cell protection by mPTP blocking (11). TSPO is involved in the cholesterol metabolism because it can interact with it *via* a long extramitochondrial C-terminal of the transmembrane 5 (TM5) helix containing a cholesterol-recognition amino acid consensus (CRAC) domain (2).

TSPO2, a paralog of TSPO1 was identified in mice, rats, and humans. It emerged from an old duplication of TSPO1. While the expression of TSPO1 is ubiquitous, TSPO2 is erythroid-specific and is located at the endoplasmic reticulum (ER), nuclear and plasma membranes (12). Similar to TSPO1, TSPO2 has the ability to bind cholesterol. Moreover, TSPO2 plays important roles in cholesterol redistribution during erythrocyte maturation (6, 13).

In this review, we focus on TSPO1 and the term “TSPO” refers to it. We will focus on the role of TSPO in the nervous system and will show its crucial role in neurogenesis, inflammation and steroidogenesis. We will also point the role of TSPO ligands able to reconstitute its basic expression

in the nervous system in order to avoid the development of neurodegenerative diseases. The input of TSPO in diagnosis as well as the use of new ligands for PET-Scan able to monitor its expression will be developed.

## **I- TSPO basal expression level is necessary to maintain homeostasis**

Tissue biodistribution of TSPO is highly conserved among species from bacteria to rodents to mammalian (14). However, the magnitude of TSPO basal expression TSPO is tissue-dependent (**Figure 1**). TSPO is found predominantly expressed in tissues synthesizing steroid hormones i.e., adrenal cortex, gonads and placenta (9) (14). In contrast, TSPO expression levels are low in the central nervous system (CNS) (15). Noteworthy, high levels of TSPO are found in steroid producing peripheral tissues other than reproductive organs, such as nasal epithelium, heart and lungs (9) (16). Within the brain, the rate of TSPO expression depends on the cell type. Higher TSPO levels are seen in glial cells, in particular microglia (17), as compared with neurons (18). Such low TSPO basal expression levels in the brain may be important for the maintenance of homeostasis. Indeed, increased TSPO levels are often concomitant with glial cell activation associated with neuroinflammation. However, the stimulation of neuronal activity increases TSPO gene and protein levels in neurons, but not in microglia or astrocytes as recently reported by Notter et al. (19).

## **II- Role of TSPO in steroidogenesis**

### a) TSPO is involved in the steroidogenesis of the CNS

One of the most debated roles of TSPO is its involvement in steroidogenesis of the CNS. Cholesterol is the main precursor of several steroid hormones, such as cortisol, aldosterone, estradiol and testosterone. Steroids are mainly synthesized by the gonads and the adrenal glands

and are well known to be involved in the development of sex secondary characteristics, reproduction, and regulation of blood pressure (20). Steroids also interact with the CNS and influence the activity and plasticity of glial cells and neurons during early development. They also have protective effects in the adult CNS (21).

The involvement of TSPO in steroidogenesis has been suggested and approved for over 30 years of research using genetic, biochemical and pharmacological approaches (22). It is important to note that discrepancies exist concerning the involvement of TSPO in steroidogenesis.

On the one hand, the importance of TSPO in steroidogenesis *via* its binding to cholesterol can be explained by the nature of each partner. The cholesterol is a highly lipophilic molecule that cannot cross the mitochondrial membrane without TSPO (**Figure 2**). Therefore, cholesterol can interact with TSPO *via* the “cholesterol recognition amino acid consensus (CRAC)” with a nanomolar affinity (23). The resulting amphiphilic molecule can diffuse into the inner mitochondrial membrane where its aliphatic tail can be cleaved by the CYP11A1 enzyme. This steroidogenic enzyme converts cholesterol into pregnenolone, which is the precursor of several steroids (24). Brain steroidogenic pathways and enzymes catalyze the production of neurosteroids. Pregnenolone, progesterone and its 5 $\alpha$ -reduced metabolites (5 $\alpha$ -dihydroprogesterone, 5 $\alpha$ -DHT and 3 $\alpha$ ,5 $\alpha$ -tetra-hydroprogesterone, 3 $\alpha$ ,5 $\alpha$ -THP also called allopregnanolone), testosterone and its-5 $\alpha$ -reduced derivatives (5 $\alpha$ -dihydrotestosterone, 5 $\alpha$ -DHT and 3 $\alpha$ ,5 $\alpha$ -tetra-hydrotestosterone, 3 $\alpha$ ,5 $\alpha$ -THP) and 17 $\beta$ -estradiol (E2) are well described as major neuroactive steroids. It is noteworthy that these steroids/enzymes are present in the human brain (25). Steroids present in the nervous system are either synthesized *de novo* from cholesterol or from a precursor arising from steroid peripheral sources through blood circulation (**Figure 2**).

It was also reported that synthetic TSPO ligands PK11195 and Ro5-4864 could promote steroidogenesis because they bind to TSPO with the same nanomolar affinity as cholesterol (26-28).

*In vitro*, it has been shown that the steroidogenic efficacy was increased in a glioma cell line as long as these ligands interact with TSPO (28, 29).

*In vivo* studies further confirmed the involvement of TSPO in steroidogenesis in the CNS. In fact, when etifoxine is injected intraperitoneally in rats at 50 mg/kg, neurosteroids synthesis was promoted. The animals showed an increase in brain pregnenolone, progesterone, 5 $\alpha$ -dihydroprogesterone and allopregnanolone concentrations by two to four-fold, compared to controls injected only with the vehicle (30). These results are consolidated by the Barron *et al.* group in TSPO-KO mice. A significant decrease in the brain production of progesterone, allopregnanolone, and dehydroepiandrosterone (DHEA) was observed in the TSPO-KO mice (31). The modulation of steroid hormones in the brain is probably the consequence of its modulation in plasma. This explanation was given by Barron *et al.* and Owen *et al.* Barron *et al.* suppressed the expression of TSPO using Cre-driven deletion techniques on C57BL/6 mice. Global steroid production levels were decreased in the KO mice (32).

Owen *et al.* performed their study on global TSPO-KO Sprague-Dawley rats using the “Zinc Finger Nuclease” technology. They also demonstrated a lethal effect due to the global deletion of TSPO: after the transfer of fertilized oocytes, the percentage of surviving animals was low. Moreover, the esterified cholesterol had not been efficiently metabolized, leading to increased lipid accumulations in steroid synthesizing tissues. Moreover, in the same paper, Owen *et al.* investigated the steroidogenic involvement of TSPO in response to stress. They focused on the TSPO single-nucleotide polymorphism (SNP) rs6971, previously described by Costa *et al.* (33).

SNP is due to a missense mutation that causes the replacement of the amino acid alanine with threonine (Ala147Thr), near the CRAC domain. This mutation was previously associated with anxiety disorders. Decreased cortisol levels after ACTH administration were observed in heterozygous and 147Thr homozygous patients, in comparison to healthy Ala147 homozygotes. This suggests that the minor allele of rs6971 SNP impairs normal ACTH-induced cortisol production in humans and reinforce the role of TSPO in CNS steroidogenesis (7) . From our point of view, the CNS steroidogenesis cannot take place without the involvement of TSPO that we argue by the convincing results of Barron et al. and Owen et al. They used the same method of analysis (HPLC/MS) to assess steroid levels methods who reflected those observed in the determination of steroids in human tissue.

#### b) TSPO is not involved in serum steroid levels

The release of four manuscripts in 2014 and 2015 did not show the involvement of TSPO in serum steroidogenesis. In fact, these research groups showed normal steroid levels *in vitro* and *in vivo* after TSPO knockout (34-37). One of these studies showed that in mice carrying TSPO deletion in Leydig cells, the production of both adrenal and gonadal steroid hormones was still normal. Moreover, testosterone production, testis development, sperm count, and fertility were not modified by TSPO deletion (37).

The other studies done *in vitro* did not depict deficits in the mitochondrial cholesterol transport in the TSPO knockout MA-10 cells (35) or in steroid hormone production, depicting that steroidogenesis is not mediated by TSPO even after exposition of TSPO knockout MA-10 cells to PK11195 (38).

The difference observed in the involvement of TSPO in serum and CNS can be explained by the fact that steroids in the nervous system may be synthesized locally or from the bloodstream.



Indeed, the majority of steroids secreted by endocrine glands, with the exception of ester sulfates, readily cross the blood-brain barrier. It is therefore difficult to distinguish between the functions of locally synthesized neurosteroids and those of circulating steroid compounds. Moreover, steroidogenesis pathways are complex and compensatory mechanisms could occur. TSPO2 expression is erythroid-specific and located in the ER, nuclear and plasma membranes whereas TSPO1 is known to be ubiquitous. Compensatory mechanisms could occur in the tissues where they are co-expressed; this could explain the divergence between the results observed in the CNS and the plasma.

### **III- Relationship between TSPO and neuroinflammation**

In the last decade, many studies have reported changes in TSPO basal expression in various forms of brain injury and diseases, in which neuroinflammation is one of the main hallmarks. At the cellular level, differential increases in TSPO rates between health and pathology are thought to be a reflection of microglial and astroglial activation, and sometimes peripheral macrophage infiltration (39) (**Figure 2**).

#### a) Modulation of TSPO expression under neuroinflammatory states

TSPO expression constitutes a biomarker of brain inflammation and reactive gliosis that can be monitored by using TSPO ligands as neuroimaging agents. Positron Emission Tomography (PET) imaging is currently accepted as an appropriate method to depict the modulation of TSPO expression (40). This non-invasive technique has allowed detecting increased expression levels of TSPO in the CNS in primarily non-inflammatory neurological disorders such as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), as well as diseases where

inflammation is the primary cause such as Multiple Sclerosis (MS) and amyotrophic lateral sclerosis (ALS) (14).

The first PET tracer used to assess TSPO expression levels in neuroinflammation was the C-labeled PK11195 (full name 1-(2-chlorophenyl)-N-[<sup>11</sup>C]methyl-N-(1-methylpropyl)-3-isoquinolinecarboxamide; [<sup>11</sup>C]-PK 11195) (41). The binding of this radiotracer was highly non-specific due to its lipophilicity, low brain uptake, and poor metabolic profile. It gave poor signal-to-noise ratio, making its quantification difficult (42, 43). Also, the half-life of this radiotracer is short (20.4 min), which is limiting for clinical trials.

Yasuno *et al.* demonstrated that [<sup>11</sup>C]DAA1106 binding to TSPO was significantly increased in brains of subjects with mild cognitive impairment (MCI) in comparison with healthy controls, thus indicating that microglial activation may occur before the onset of AD (44). However, this needs further confirmation. [<sup>18</sup>F]DAA1106 is another TSPO radioligand derived from the phenoxyarylacetamide class. Autoradiography studies performed on postmortem AD brains using [<sup>11</sup>C]DAA1106 showed a significantly higher binding in the hippocampus, the temporal and parietal cortices, the basal ganglia and the thalamus, thus demonstrating that [<sup>11</sup>C]DAA1106 is an appropriate marker of activated microglia with upregulated TSPO in AD (45).

[<sup>18</sup>F]FEDAA1106 (full name (N-(5-fluoro-2-phenoxyphenyl)-N-(2-(2-fluoroethoxy)-5-methoxybenzyl) acetamide)), also derived from the phenoxyarylacetamide class, has been recently studied as a potential tool to visualize TSPO *in vivo*. The radioligand showed a good binding affinity to TSPO (0.078 nmol/L) and high lipophilicity ( $\log D = 3.81$ ) (46). However, [<sup>18</sup>F]FEDAA1106 cannot be used to monitor microglial activation in AD and MS. Its brain distribution volumes ( $V_T$ ) and the binding potential (BP) are not significantly different between AD and healthy and between patients with multiple sclerosis (MS) and healthy individuals (47).

Vinpocetine is a vinca alkaloid compound conceived as a PET tracer labeled with  $^{11}\text{C}$  ( $[^{11}\text{C}]$  vinpocetine) with a high affinity to TSPO (16). No significant changes in radioactivity were seen between AD and healthy control brains (48), or between the binding potential of  $[^{11}\text{C}]$  vinpocetine and  $[^{11}\text{C}]$ -PK 11195 (49).

The new developed TSPO radioligands also may have limitations due to the variability of TSPO binding potential (BP), resulting from a single nucleotide polymorphism (*rs6971*) in the *Tspo* gene (50), thus affecting the binding affinity of TSPO to its ligands. The development of third-generation PET-tracers, not affected by this substitution, might overcome this problem.

Of great interest is the use of TSPO PET scan in clinical trials. The radiotracer  $[^{11}\text{C}]$ PBR28 is currently used to measure post-stroke brain inflammation in patients aged 18-85 years and undergoing a recent moderate ischemic stroke (within the last 10 days) (ClinicalTrials.gov: NCT03662750). It is also tested to study changes in the brain after a traumatic brain injury (TBI) as compared to healthy controls, and the study is currently in phase 2 (ClinicalTrials.gov: NCT01547780). While the work cited above deals with global neuroinflammation, microglial activation is precisely assessed by using  $[^{18}\text{F}]$ PBR111 in patients with MS as compared to healthy controls (ClinicalTrials.gov: NCT01428505). PET-scanning is not only informative in acute brain injury, but also in neurodegenerative diseases.

TSPO PET imaging is also a reliable biomarker in animal models of neurodegenerative diseases. Indeed, the up regulation of TSPO levels is confirmed in two mouse models of AD. In APP/PS1dE9 transgenic mice, the microglia/macrophage marker Iba1 colocalizes with high TSPO levels in all brain regions studied, i.e. cortex, hippocampus, cerebellum striatum, except the thalamus (51). In 5XFAD mice, which carry five familial AD mutations, TSPO was highly expressed in A $\beta$  plaques associated microglia, a typical feature of AD (9). Therefore, TSPO

expression in the brain is linked to activated state of microglia and infiltrating macrophages (39). In support of this statement, two *in vitro* studies demonstrated that the absence of TSPO accelerates the evolution of neuroinflammation. The first study demonstrates that BV2 microglia murine cells transfected with TSPO-targeting siRNA have increased levels of pro-inflammatory mediators such as interleukin 6 (IL-6) and tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ). In addition, the authors show that TSPO-knockdown reduced the anti-inflammatory effects of PK1195 and Ro5-4864 (52). The second study reports that the knockdown of TSPO in RAW264.7 macrophage murine cells results in an increase of the hemin-induced release of pro-inflammatory cytokines (53). Since chronic inflammation could lead to the development of neurodegenerative diseases (54), we postulate that the absence of TSPO could be an important risk factor in the progression of such pathologies. However, it is important to note that discrepancies in the role of TSPO in neuroinflammation exist between species, particularly between humans and rodents, despite the sequence similarity. Owen et al. showed that TSPO expression levels were not increased in human adult microglia (HAM) neither after a classic pro-inflammatory stimulus (IFN $\gamma$ /LPS), nor after a stimulus of reparative activation (IL4/IL13). Surprisingly, TSPO levels were decreased after the pro-inflammatory activation of human monocyte-derived macrophages (MDM). In contrast, the pro-inflammatory stimuli caused increases in TSPO levels in rodent MDM and primary monocytes (55).

#### b) Dampening of neuroinflammation by TSPO ligands

Two TSPO ligands, 2-Cl-MGV-1 ( (2-(2-chlorophenyl) quinazolin-4-yl dimethylcarbamate)) and MGV-1 (2-phenylquinazolin-4-yl dimethylcarbamate), have been shown to contribute to the attenuation of neuroinflammation in lipopolysaccharide (LPS)-treated microglia BV-2 cells, by

decreasing their IL-1 $\beta$ , IL-6, tumor necrosis factor alpha (TNF- $\alpha$ ) and interferon gamma (IFN $\gamma$ ) (56). In the same cellular model of activated LPS-treated microglia BV-2 cells, two other TSPO ligands, 2-U-M6V-1 (2-(2-chlorophenyl) quinazolin-4-yl dimethylcarbamate) and M6V-1 (2-phenylquinazolin-4-yl dimethylcarbamate) also display anti-inflammatory effects. The underlying mechanisms involve the decreased production of interleukin-1 $\beta$  (IL-1 $\beta$ ), IL-6, (TNF- $\alpha$ ), and (IFN- $\gamma$ ), as well as an inhibition of NF- $\kappa$ B p65 activation (56).

Moreover, TSPO ligand etifoxine (Stresam®) enhanced the neurite outgrowth in PC12 cells and after nerve injury (57). Etifoxine also promoted neuroprotection in a mouse model of intracerebral hemorrhage by reducing leukocyte infiltration into the brain and microglial production of IL-6 and TNF- $\alpha$ , thus attenuating cell death (58). PK11195, another high affinity TSPO ligand, promotes neuroprotection by dampening neuroinflammation in male SPF C57/BL6 mice treated with LPS injections (59). PK11195 was found to enhance neuronal survival by inhibiting microglial activation in excitotoxic injured rats after quinolinic acid injection into the striatum (60). Taken together, these results depict the important outcomes of TSPO ligands for preventing neuroinflammation.

#### **IV- TSPO and Reactive Oxygen Species**

Reactive oxygen species (ROS) are free radicals derived from molecular oxygen (61). When overproduced, they lead to oxidative stress which can damage DNA, proteins, and lipids. This could result in the induction of several neurodegenerative diseases. Given the fact that mitochondria are the main source of ROS production (62), that ROS overproduction is deleterious for cells and that TSPO is located on the OMM, understanding how TSPO affects ROS levels needs to be addressed. It is known that cholesterol entry into the mitochondria

through TSPO regulates the activity of the ATP synthase which is responsible for ROS production (**Figure 3**). This results in the oxidation of cardiolipin, along with mPTP opening followed by ATP and Ca<sup>2+</sup> release and mitochondrial membrane depolarization. Cytochrome c is then free to go into the cytosol due to the opening of the Bax/Bak channel after depolarization, and thus initiating apoptosis (63).

Moreover, it is well established that mitophagy takes place in damaged mitochondria. Mitophagy consists of selectively delivering damaged mitochondria for lysosomal degradation through the phosphatase and tensin homolog (PTEN)-induced putative protein kinase 1 (PINK1) and PARK2 pathway (PINK1-PARK2 pathway) (64). PINK1 selectively accumulates on the outer depolarized mitochondrial membrane and recruits the cytosolic E3 ubiquitin ligase (PARK2) (65). PARK2 then ubiquitinates the proteins located on the outer mitochondrial membrane and activates sequestosome 1 (SQSTM1), which leads to autophagy (66). It has been suggested that VDAC1–interacts with PARK2 and plays a role in its recruitment and function through the mitophagy process. When the ratio of TSPO/VDAC1 expression increases, ROS production increases, thus decreasing the efficiency of the ubiquitin-dependent mitophagy (67). In mouse embryonic fibroblasts, the increase in TSPO expression positively correlates with ROS production which modulates the levels of the autophagy marker LC3B-II, and alters the ubiquitination of proteins involved in mitophagy (67). Therefore, one can conclude that modulation of mitophagy by TSPO is independent from cholesterol binding, and mainly relies on the interactions with the VDAC1.

In the CNS, the main molecular compound controlling ROS is the NADPH oxidase (NOX2). It has been suggested by Guilarte et al. that interactions between TSPO and NOX2 (NOX2), which produces ROS, may activate the transcription factor Nrf2 (68). This transcription factor is

implicated in redox homeostasis and antioxidant responses. Such interactions may work together in controlling ROS release in microglia, especially under chronic neuroinflammatory conditions (68).

The link between TSPO and ROS is well-documented *in vitro*. TSPO knockdown significantly increases ROS production in BV-2 microglia (69). TSPO ligands etifoxine and XBD173 suppress ROS production and increase antioxidant capacity in RF/6A chorioretinal cells treated with oxidized low-density lipoprotein oxLDL—an inducer, of ROS production (70, 71). In addition, PK11195 decreases basal ROS generation in Balb/c mice primary peritoneal macrophages (72). Ro5-4864 reduces ROS production in glucose-deprived astrocytes, compared to controls (73). Taken together, the previous findings highlight the cellular protective role of TSPO ligands against ROS damages in the CNS, and focus on the regulatory role of TSPO in the modulation of ROS expression levels.

## **V. TSPO, neuroprotection and neuroregeneration**

### a) Effects of TSPO ligands on neuron survival

TSPO ligand Ro5-4864 (full name (7-chloro-5-(4-chlorophenyl)-1,3-dihydro-1-methyl-2H-1,4-benzodiazepine-2) promotes neuroprotection, as shown by its dampening outcome on pain in rats with traumatic brain injuries, and its enhancing effect on neurons survival (74). Similar results were observed by Ma *et al.* after sciatic nerve lesions in Sprague-Dawley rats (75). In addition, Ro5-4864 protected cultured neonatal DRG from cell death, but not adult neurons (76). TSPO ligand etifoxine (Stresam®) enhanced the neurite outgrowth in PC12 cells (57). Also, the administration of Ro5-4864 *in vivo* enhanced spinal neonatal motoneurons survival after sciatic nerve axotomy (76).

Recently, GRT-X (N-[(3-fluorophenyl)-methyl]-1-(2-methoxyethyl)-4-methyl-2-oxo-(7-trifluoromethyl)-1H-quinoline-3-carboxylic acid amide), a novel drug which can activate both the voltage-gated potassium channels of the Kv7 family and TSPO, has been shown to enhance survival and accelerate regrowth of sensory and motor neurons after severe crush lesion of cervical spinal nerves in rats (a rat model of chronic neuropathic pain) (77). Further studies are needed to assess the GRT-X efficiency in human neuronal survival.

#### b) Neuroprotective effects of TSPO ligands after nerve injury

TSPO ligands exhibit a wide range of neuroprotective activities. PK11195 enhances neuronal survival by inhibiting microglial activation in excitotoxic injured rats after quinolinic acid injection into the striatum (60). Another TSPO ligand, the SSR180575 (full name, indoleacetamide-derived 7-chloro-N,N,5-trimethyl-4-oxo-3-phenyl-3,5-dihydro-4H-pyridazino[4,5-b] indole-1-acetamide, SSR180575), promotes functional recovery in young rats following facial nerve injury (78). Following traumatic cortical brain damage in rats, etifoxine is neuroprotective through several mechanisms including reduction of apoptosis, enhancement of neuronal survival, restoration of mitochondrial oxidative phosphorylation and is associated with improved cognitive outcomes (79, 80). In the model of chronic neuropathic pain involving cervical spinal nerve lesion, GRT-X accelerates the rate of recovery of normal function in sensory and motor neurons, indicative of its efficient role in promoting neuroregeneration (77).

#### c) Neuroprotective effects of TSPO ligands in neurodegenerative diseases

With regards to AD, Ro5-4864 reverses  $\beta$ -amyloid accumulation, gliosis, and behavioral impairment, in the triple transgenic (3xTgAD) mouse model of AD (81). Similarly, PK11195 attenuates  $\beta$ -amyloid pathology and impaired behaviors in aged 3xTg-AD female mice (82) as well as in SPF C57/BL6 male mice injected with LPS (59). Two new TSPO ligands, named 2a



and 2b, also exert beneficial effects in a cellular model of AD- related tauopathy presenting mitochondrial impairments, by increasing ATP synthesis mitochondrial membrane potential, as well as pregnenolone synthesis (83). Based on these data, TSPO ligands behave as potential anti-alzheimer agents targeting several cellular mechanisms.

On the other hand, the TSPO ligand EMAPUNIL® (XBD173) displays beneficial effects on the well-known MPTP-induced mouse model of PD by preserving striatal dopamine depletion and preventing motor deficits (84). Treatment with XBD173 also improves clinical and neuropathological features (oligodendroglial regeneration and decreased peripheral immune cell infiltration of the spinal cord) in the mouse model of MS, known as experimental autoimmune encephalomyelitis (EAE), inducing an inflammatory demyelination (85). **Table 1** summarizes the activities of TSPO ligands and their beneficial effects on neuroinflammation and CNS pathologies.

## Conclusion

As evidenced in this review, TSPO plays a key role in the nervous system. It maintains homeostasis and regulates intramitochondrial cholesterol transfer and its cleavage into pregnenolone, the first and major pathway for neurosteroids production. Proofs of TSPO involvement in brain steroidogenesis are partly related to the TSPO ligand-induced stimulation of endogenous steroid levels in the rodent brain, under physiological or pathological conditions. Several TSPO targeted molecules, tested in preclinical studies, have proved their efficacy as neuroprotective, anti-inflammatory and regenerating agents, and in counteracting damages caused by ROS in the nervous system. In the meantime, TSPO has become an important target in the follow-up of several neurodegenerative diseases such as AD, HD, and PD using PET TSPO radioligand imaging. To conclude, ongoing advanced clinical trials with three TSPO ligands

(etifoxine, XBD173, PK11195) will definitely give their potential therapeutic efficiencies in CNS and PNS diseases.

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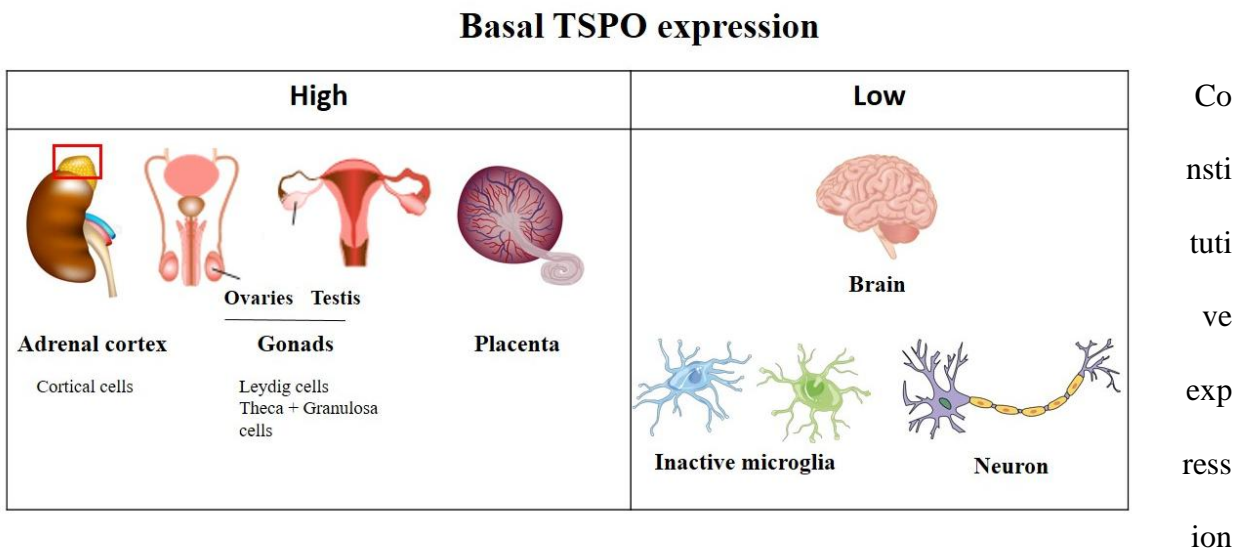
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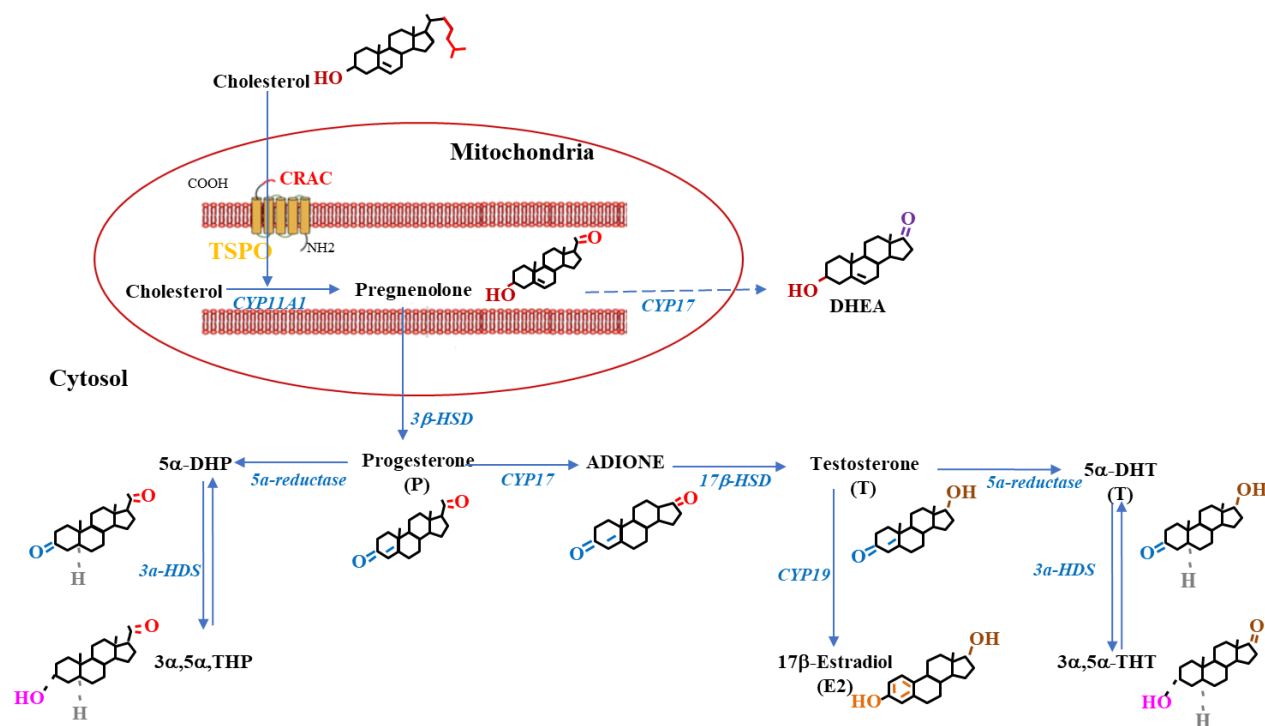
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**Figure 1.** Basal TSPO expression levels in tissues and cells.



of TSPO in steroidogenic tissues. TSPO levels are higher in peripheral tissues, such as adrenal cortex, gonads and placenta, compared to the brain. The red square represents the adrenal cortex.

**Figure 2.** Role of TSPO in steroidogenesis.

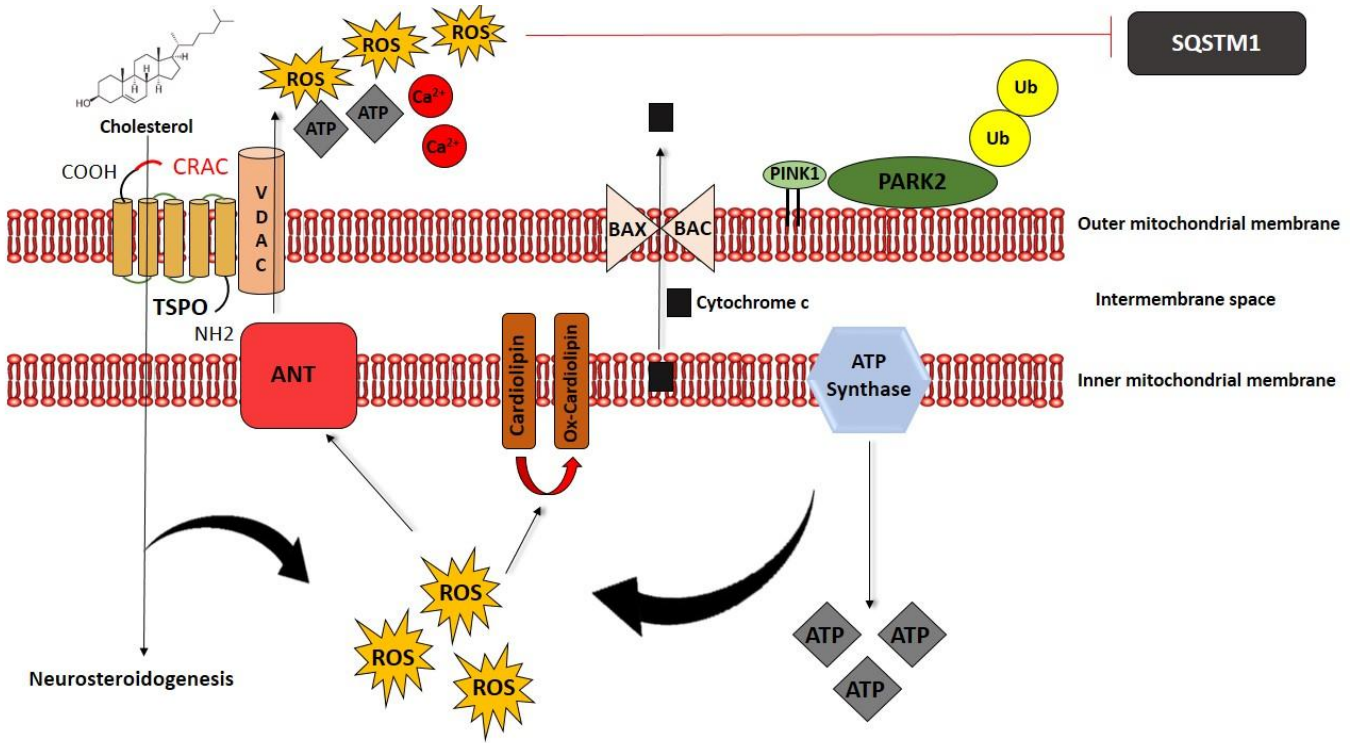


Cholesterol binding to the C-terminal of TSPO allows its transfer from the OMM to the IMM where CYP11A1 cleaves its aliphatic tail, resulting in the biosynthesis of pregnenolone. Pregnenolone is the precursor of all steroids, such as progesterone and its 5 $\alpha$ -reduced derivatives, testosterone and its 5 $\alpha$ -reduced metabolites, and 17 $\beta$ -estradiol, which are major neuroactive steroids

**Abbreviations:** CYP11A1: cytochrome P450<sub>scc</sub> (scc: side chain cleavage); 3 $\beta$ -HSD: 3 beta-hydroxysteroid dehydrogenase  $\Delta^5$ – $\Delta^4$  isomerase; CYP17: cytochrome P450<sub>C17</sub> (17 $\alpha$ -hydroxylase/17,20 lyase); 17 $\beta$ -HSD: 17 $\beta$ -hydroxysteroid dehydrogenase; CYP19: cytochrome P450 aromatase. The dashed arrow indicates that the synthesis of dehydroepiandrosterone (DHEA) is elusive in rodents' nervous system whereas it occurs in human brains.



**Figure 3.** Crosstalk between TSPO and ROS.



Cholesterol enters into the mitochondria through TSPO. This process enables neurosteroidogenesis on the one hand, and the production of ROS on the other hand. Also, the production of ATP by the ATP Synthase produces ROS. Cardiolipin is oxydated by ROS which permits the opening of the mPTP. ATP and calcium can be released, leading to the depolarization of the mitochondrial membrane. The depolarization of the membrane opens the Bax/Bak channel and cytochrome c can be released into the cytosol, initiating apoptosis. Moreover, the increase in the expression of TSPO increases the production of ROS which inhibits mitophagy by blocking the ubiquitination of VDAC by the PINK1/PERK2 pathway and the SQSTM1 recruitment.

**Table 1.** Effects of TSPO ligands in neurodegenerative diseases and nerve injuries.

<b>TSPO Ligand</b>	<b>Pathology involved</b>	<b>Role</b>	<b>Reference</b>
Etifoxine (STRESAM <sup>®</sup> )	<ul style="list-style-type: none"> <li>- Experimental autoimmune encephalomyelitis (mice)</li> <li>- Lesion of the sciatic nerve (freeze injury in rats)</li> <li>- Intracerebral hemorrhage (mice)</li> </ul>	<ul style="list-style-type: none"> <li>- Delayed clinical symptoms, modulated inflammatory responses, and beneficial effects after neural injury</li> <li>- Axonal regeneration and speed up of functional recovery</li> <li>- Reduction of leukocyte infiltration into the brain and decrease of microglial production of IL-6 and TNF-<math>\alpha</math></li> </ul>	(57, 58, 85)
SSR180575	Facial nerve injury (rats)	Promotion of functional recovery	(78)
2-U-M6V-1 and M6V-1	Inflammation due to microglia activation by lipopolysaccharide (LPS)	Decrease of neuroinflammation and reduction of the production of interleukin-1 $\beta$ (IL-1 $\beta$ ), IL-6, cytokines tumor necrosis factor- $\alpha$ (TNF- $\alpha$ ), and interferon gamma (IFN- $\gamma$ )	(56)
Ro5-4864	<ul style="list-style-type: none"> <li>- Peripheral nerve injury (Rodents)</li> <li>- Alzheimer's disease (mice)</li> </ul>	<ul style="list-style-type: none"> <li>- Neuroprotection and pain alleviation</li> <li>- Protection of neonatal spinal cord motor neurons from death after sciatic nerve axotomy</li> <li>- Neuroprotective effect on adult facial motor neurons after axotomy</li> <li>- Decrease of A<math>\beta</math> plaques accumulation in the hippocampus, decrease of gliosis, behavior improvement</li> </ul>	(75, 81, 86)
PK11195	<ul style="list-style-type: none"> <li>- Alzheimer's Disease (AD) (female 3xTg-AD mice)</li> <li>- Excitotoxic injury (injection of quinolinic acid into the rat striatum)</li> </ul>	<ul style="list-style-type: none"> <li>- Improvement in behavior and small reduction in soluble and deposited markers of <math>\beta</math> amyloid plaques</li> <li>- Increase of neuronal survival and inhibition of microglial activation</li> </ul>	(82) (87)
XBD-173 (EMAPUNIL <sup>®</sup> )	Parkinson's Disease (female mice)	Amelioration of degeneration of dopaminergic neurons, preservation of striatal dopamine metabolism, prevention of motor dysfunctions, and decrease of cellular stress and apoptosis	(84)