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Translocator protein and steroidogenesis

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Two interesting papers by Barren et al. and Owen et al. have been very recently published in Biochemical Journal, reporting the role of translocator protein (TSPO) in steroidogenesis. The involvement of TSPO in the steroid biosynthesis has been suggested by 30 years of researches, using biochemical, pharmacological and genetic experimental approaches. In the last 3 years, however, the TSPO involvement in steroidogenesis has been intensively and profoundly discussed. Using *in vivo* genetic manipulations aimed at deleting TSPO, some researchers have excluded its role in steroid production. Other research groups, using similar genetic manipulation techniques, have presented different results, corroborating the role of TSPO in steroidogenesis, in particular, when hormonal stimulation occurs. In this scenario, the publications by Barron et al. about 'Steroidogenic abnormalities in translocator protein knockout mice and significance in the aging male' and by Owen et al. about 'TSPO mutations in rats and a human polymorphism impair the rate of steroid synthesis' are part of this debate and provide further and more accurate information supporting the importance of TSPO as a steroidogenesis regulator.

Barron et al. [1] and Owen et al. [2] have recently published two interesting papers in the Biochemical Journal, reporting the role of translocator protein (TSPO) in steroidogenesis.

Steroids are four-fused-ring organic compounds with crucial biological functions. These molecules are essential components of cell membranes and are involved in cellular signalling and hormonal control. They are ubiquitously synthesized, mainly in the adrenal glands and gonads. They are present in the circulatory system and other organs, including the brain. Among various biological effects exerted by steroids (i.e. immunosuppression, blood pressure, and sex secondary characteristics), an important action is the proposed rapid control of the central nervous system excitability [3].

The ubiquitous TSPO shows the highest expression levels in steroid-synthesizing tissues. Converging data have suggested that it is involved in the translocation of cholesterol into the mitochondria, the first rate-limiting step of steroidogenesis. In this line, most of the studies investigating the knockout or silencing of TSPO in various cellular steroidogenic models have shown a markedly reduced steroidogenic capacity or, in some cases, the complete inability to produce steroids (both under baseline and following hormone stimulation conditions) [4–8]. In addition, the results obtained through in vitro experiments, aiming to overexpress the various components of the steroidogenic metabolome (including TSPO), have highlighted the important role of TSPO in steroidogenesis [9]. The involvement of TSPO in such an important biological function has been suggested for 30 years of research using not only genetic in vitro approaches but also biochemical and pharmacological ones [10]. In 2014, however, this topic was questioned following the publication of three manuscripts by two independent research groups. In these papers, in vivo genetic studies have excluded a steroidogenic role for TSPO, based on normal steroid levels found in the plasma of global TSPO knockout mice [11,12] and in peripheral TSPO-lacking steroidogenic tissues [13]. In 2015, a regular steroid baseline production, but a reduced ability to produce steroids following adrenocorticotropic hormone (ACTH) stimulation, have been demonstrated using a conditional TSPO knockout mouse model [14]. Furthermore, in global TSPO knockout models, controversial data concerning the essential role for TSPO in embryonic development have been reported [11,12,14,15].

Experimental models, analytical techniques, and steroid values obtained by the different research groups are summarized in Table 1. In the table, the used techniques and the obtained results by Barron et al. and Owen et al. are reported too.

In 2018, Barron et al. [1] in the paper entitled ‘Steroidogenic abnormalities in translocator protein knockout mice and significance in the aging male’ found a reduced level of global steroid

production under basal conditions in TSPO knockout mice. Moreover, they have underlined the crucial role of TSPO in maintaining androgen production during ageing. All of these evaluations have been obtained using rodent (C57BL/6 mice) in vivo genetic manipulations aimed at eliminating the TSPO gene using Cre-driven deletion strategies to obtain global TSPO knockout mice, as previously used by the recent literature [11,12]. Morohaku et al. [13] and Fan et al. [14] have used the same strategy to obtain the TSPO deletion in specific tissues.

Owen et al. [2] in the paper entitled 'TSPO mutations in rats and a human polymorphism impair the rate of steroid synthesis' in TSPO knockout rats have demonstrated: (i) a reduced ability to produce steroids in response to ACTH treatment; (ii) a low percentage of live animals following the transfer of fertilized oocytes, suggesting a lethal effect exerted by the global deletion of TSPO; and (iii) an increase in lipid accumulation in steroidogenic tissues, suggesting that the esterified cholesterol had not been efficiently metabolized. These authors have generated global TSPO knockout Sprague–Dawley rat models using the Zinc Finger Nuclease technology [2].

As reported in Table 1, notably, both Owen et al. and Barron et al. have used the most sensitive highperformance liquid chromatography/mass spectrometry technique (HPLC/MS) for the evaluations of steroid levels in addition to other common used techniques such as enzyme-linked immunosorbent assays (ELISA) and radioimmunoassys (RIA).

Different steroids have been evaluated in the examined papers: ALDO, CORT, DHT, P4, P5, and T by Barron et al. [1], and T and CORT by Owen et al. [2]. Same or different steroids have been assessed by the other groups: E, oestradiol, P4, T, CORT, and ALDO by Tu et al. [11]; P5, E, and T by Morohaku et al. [13]; and P5 was assessed by Banati et al. [12].

In the works of Barron et al. and Owen et al., the comparisons of plasma steroid levels between genotype groups (TSPO+/+ and TSPO−/−) are based on statistically robust data.

After a critical examination of the related literature and the available in vivo genetic data, the approach adopted by Barron et al. has yielded accurate experimental results. Differently by other works, their research has evaluated not only the most common steroids produced by gonads and adrenal glands but also the overall steroidogenic capacity in terms of measuring the cholesterol metabolites generated in a series of sequential reactions with a 1 : 1 stoichiometry. Although TSPO has not been demonstrated to be critical for maintaining the 'survival essential steroid levels', their findings have suggested that TSPO deficiency leads to a great variation in the steroidogenic flow, causing a reduced global production of steroids, especially cortisol and progesterone [1].

The results by Owen et al. support the steroidogenic involvement of TSPO in the stress response. The levels of the steroid mainly produced by the adrenal gland-derived corticosterone following ACTH stimulation were significantly reduced in the global TSPO knockout models with respect to control, confirming the previous data obtained in a conditional TSPO knockout model [14].

To extend the knowledge on the role of TSPO steroidogenic regulation in human stress response, Owen et al. [2] have focused attention on the TSPO single-nucleotide polymorphism (SNP) rs6971, previously associated with anxiety disorders [16]. This polymorphism is a missense mutation that replaces the amino acid alanine 147 with threonine (Ala147Thr), which lies in the proximity of the interaction domain with cholesterol. In healthy individuals, Owen et al. have documented a correlation between the genotype and plasma cortisol levels following ACTH administration. Heterozygotes and 147Thr homozygotes have lower cortisol levels than the Ala147 homozygotes (gene–dose effect), suggesting that the minor allele of rs6971 SNP in humans impairs normal ACTH-induced cortisol production.

From all these data, the previously suggested thesis by various authors that 'TSPO function is not essential for steroid hormone biosynthesis' appears to be based on partial analysis, especially in light of the most recent results shown by Barron et al. [1] and Owen et al. [2]. Considering the complexity of the steroidogenesis pathways and the potential compensation mechanisms caused by the inactivation of a protein, it seems that more data derived from both different accurate experimental strategies and robust statistical analyses are fundamental to draw reliable conclusions.

Finally, the data obtained by Owen et al. and Barron et al. provide new insights into the complex relation between TSPO and steroidogenesis, contributing to the clarification of some points regarding the apparent discrepancies among in vivo genetic manipulation studies.

Abbreviations

ACTH, adrenocorticotropic hormone; Ala147Thr, amino acid alanine 147 with threonine; ELISA, enzyme-linked immunosorbent assays; RIA, radioimmunoassys; SNP, single-nucleotide polymorphism; TSPO, translocator protein.

Competing Interests

The Authors declare that there are no competing interests associated with the manuscript.

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Table 1 *In vivo* genetic models, analytical techniques, and steroid values obtained by different research groups

				Steroid levels (*n*; L)						
	***In vivo* model**	**Analytical technique**	**Basal or stimulation**	**P5**	**P4**	**E**	**DHT**	**T**	**CORT**	**ALDO**
Barron et al. [1]	GK C57BL/6 mouse	- LC–MS/MS (nM)	Basal level in young animals	WT = 10; 0.6 KO = 10; 0.3	WT = 10; 4.5 KO = 10; 1.8	/ /	WT = 10; 0.15 KO = 10; 0.6	WT = 10; 1 KO = 10; 3.8	WT = 10; 220 KO = 10; 120**	WT = 10; 1 KO = 10; 1
			Basal level in old animals	WT = 10; 0.9 KO = 10; 0.7	WT = 10; 6 KO = 10; 3.8	/ /	WT = 10; 0.08 KO = 10; 0.015*	WT = 10; 0.8 KO=10; 0.5*	WT = 10; 450 KO = 10; 400	/ /
	CaMKIIa-Cre									
Owen et al. [2]	GK (mutation) Rat ZFN	- RIA - ELISA - LC–MS (ng/ml)	Basal level ACTH stimulation	/ / / /	/ / / /	/ / / /	/ / / /	WT = 5; 5.5 KO=5, 1.5** / /	WT = 12; 25/10 KO = 12; 10/12 WT = 12; 75/40 KO = 12; 10/20**	/ / / /
Fan et al. [14]	CK (adrenal cortex and gonads) C57BL/6 mouse Amhr2-Cre Nr5α1-Cre	- EIA - RIA (ng/ml)	Basal level ACTH stimulation CG stimulation 	/ / / / / /	/ / / / / /	/ / / / / /	/ / / / / /	WT = 10; 10 KO = 10; 9 / / WT = 10; 35 KO = 10; 35	WT = 10; 45 KO = 10; 43 WT = 10; 100 KO = 10; 45* / /	/ / / / / /
Tu et al. [11]	GK C57BL/6 mouse Amhr2-Cre	- RIA (CORT, T and P4: ng/ml ALDO and E: pg/ml)	Basal level ACTH stimulation CG stimulation 	/ / / / / /	WT = 11; 3 KO = 15; 5 / / / /	WT = 12; 20 KO = 15; 25* / / / /	/ / / / / /	WT = 26; 0.5 KO = 25; 0.8 / / WT = 6; 32 KO = 7; 32	WT = 24; 320 KO = 24; 260 WT = 10; 780 KO = 10; 720 / /	WT = 7; 250 KO = 7; 180 / / / /
Morohaku et al. [13]	CK (gonads) C57BL/6 mouse Amhr2-Cre	- ELISA - RIA (E: pg/ml P5, T: ng/ml)	Basal level CG stimulation 	WT = ?; 4.8 KO = ?; 3.5 / /	/ / / /	WT = ?; 21.5 KO = ?; 17.5 / /	/ / / /	WT = 19; 0.9 KO = 22; 0.3 WT = 7; 30 KO = 7; 31	/ / / /	/ / / /
Banati et al. [12]	GK C57BL/6 mouse Amhr2-Cre	- ELISA (ng/ml)	Basal level	WT = 4; 140 KO = 4; 143	/	/	/	/	/	/

Abbreviations: GK: global knockout; CK: conditional knockout; ZFN: Zinc Finger Nuclease technology; ALDO: aldosterone; CORT: corticosteroid; DHT: dihydrotestosterone; E: oestradiol; P4: progesterone; P5: pregnenolone; T: testosterone; ELISA: enzyme-linked immunosorbent assay; LC–MS/MS: liquid chromatography–mass spectrometry/mass spectrometry; RIA: radioimmunoassay; CG: chorionic gonadotropin; ACTH: adrenocorticotropic hormone.
WT: $TSPO^{+/+}$; KO: $TSPO^{-/-}$; *n*: number of animals; L: steroid level value; ?: data not reported.
*$P < 0.05$ or **$P < 0.01$ vs. WT.