



Effect of antipsychotics on mitochondrial bioenergetics of rat ovarian theca cells



Ekramy Elmorsy^a, Ayat Al-Ghafari^b, Amal Misbah Aggour^c, Soaad Mohamed Mosad^a, Raheela Khan^d, Saad Amer^{d,*}

^a Department of Forensic Medicine and Clinical Toxicology, Faculty of Medicine, Mansoura University, Egypt

^b Biochemistry Department, Faculty of Science, King Abdulaziz University (KAU), Jeddah, Saudi Arabia

^c Clinical Pathology specialist, Ministry of Health, Egypt

^d Division of Medical Sciences and Graduate Entry Medicine, School of Medicine, University of Nottingham, Royal Derby Hospital, Uttoxeter Road, Derby DE22 3DT, UK

HIGHLIGHTS

- This study investigated the effects of four antipsychotics (APs) on mitochondrial bioenergetics and steroidogenesis of rats isolated ovarian theca interstitial cells (TICs) as a possible mechanism of reproductive toxicity.
- The APs used in this experiment include chlorpromazine (CPZ) haloperidol (HAL), risperidone (RIS) clozapine (CLZ).
- All four APs seem to inhibit mitochondrial bioenergetics and steroidogenesis in rat's TICs.
- These findings support the hypothesis that APs-induced reproductive toxicity may be through mechanisms involving mitochondrial insult.

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ABSTRACT

Background: Antipsychotics (APs) are widely prescribed drugs, which are well known to cause reproductive adverse effects through mechanisms yet to be determined. The purpose of this study was to investigate the effect of antipsychotics on mitochondrial bioenergetics of rat ovarian theca cells as a possible mechanism of reproductive toxicity.

Methods: Isolated rat theca interstitial cells (TICs) were treated with two typical (chlorpromazine [CPZ] and haloperidol [HAL]) and two atypical APs (risperidone [RIS] and clozapine [CLZ]). The effects of these APs on TICs bioenergetics (ATP content, mitochondrial complexes I and III activities, oxygen consumption rates (OCRs), mitochondrial membrane potential (MPP) and lactate production) and on steroidogenesis (androstenedione and progesterone synthesis) were investigated.

Results: All APs resulted in a concentration-dependent decrease in the ATP content of TICs. All APs at their estimated IC₅₀s (6 μM, 21 μM, 35 μM and 37 μM for CPZ, HAL, CLZ and RIS respectively) significantly decreased TICs OCRs ($p < 0.0001$), MPP ($p < 0.0001$) and significantly ($p = 0.0003$) inhibited mitochondrial complex I activity. Only typical APs inhibited complex III ($p = 0.005$). Also, APs at IC₅₀s increased TICs lactate production to varying degrees. All APs used at their IC₅₀s significantly inhibited progesterone ($p = 0.0022$) and androstenedione ($p = 0.0027$) production. Only CPZ was found to inhibit these hormones at the low concentration (1 μM).

Conclusion: All four antipsychotics seem to inhibit mitochondrial bioenergetics and steroidogenesis in rat's ovarian theca cells. These findings support the hypothesis that APs-induced reproductive toxicity may be through mechanisms involving mitochondrial insult. Further research is required to establish the link between APs-induced mitochondrial dysfunction and disordered steroidogenesis.

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1. Introduction

Antipsychotics (APs) are well known for their toxicity, which affects almost all systems of the body resulting in a wide range of

* Corresponding author.

E-mail address: Saad.amer@nottingham.ac.uk (S. Amer).

manifestations such as extra-pyramidal symptoms, weight gain and reproductive dysfunction. Chlorpromazine (CPZ) has been shown to inhibit ovulation in rats via suppression of the LH surge (Everett and Tyrey, 1982). Haloperidol (HAL) has been shown to lower ovarian and uterine weights and to inhibit ovulation in rats (Boris et al., 1970). Antipsychotic-induced hyperprolactinaemia has been strongly suggested as a mechanism for these reproductive dysfunctions. Several studies have reported an association between hyperprolactinaemia and reproductive dysfunction in women receiving APs (Ghadirian et al., 1982; Meltzer et al., 1983; Bargiota et al., 2013). However, other studies reported no association between hyperprolactinaemia and reproductive dysfunction during AP therapy (Canuso et al., 2002). Therefore, the mechanisms explaining the reproductive adverse effects of APs remain largely uncertain.

Several APs, such as haloperidol (HAL), chlorpromazine (CPZ), thiothixine and clozapine (CLZ), have been found to inhibit mitochondrial respiratory enzyme complexes, which have a crucial role in Oxidative phosphorylation cycle for the synthesis of adenosine triphosphates (ATP) (Burkhardt et al., 1993). Inhibited respiratory enzyme complexes have been found to induce damage to the mitochondria via the release of reactive oxygen species (ROS) (Wei et al., 1998 and Harper et al., 2004; Guo et al., 2013). This mitochondrial damage has been suggested as a possible mechanism of AP-induced extrapyramidal side effects such as tardive dyskinesia (Goff et al., 1995 and Casademont et al., 2007). This hypothesis, which is widely accepted, has been supported by several studies reporting a correlation between the tardive dyskinesia and mitochondrial inhibition during APs treatment and production of ROS (Grenell et al., 1955; Guth and Spirtes, 1964; Gallagher et al., 1965; Elkashef and Wyatt, 1999).

In this study, we hypothesized that mitochondrial dysfunction is the underlying mechanism of AP-induced reproductive adverse effects. Ovarian theca interstitial cells (TICs) were isolated from rats and used as a cell model to test the effects of APs. The primary aim of this study was to investigate the cellular and functional effects of two typical APs (haloperidol [HAL], chlorpromazine [CPZ]) and two atypical APs (risperidone [RIS] and clozapine [CLZ]) on mitochondrial bioenergetics and on steroidogenesis of TICs from rats.

2. Materials

2.1. Chemicals and media

All chemicals and reagents including the APs were purchased from Sigma-Aldrich (Poole, UK) unless stated otherwise. Two typical (CPZ and HAL) and two atypical (RIS and CLZ) APs were obtained for this study. All stock solutions of drugs were made in DMSO (vehicle) then diluted further in media as required. Media and their additives were purchased from Gibco (Grand Island, NY). The adenosine triphosphate (ATP) assay and lactate colorimetric assay kits were purchased from Abcam (Cambridge, MA, USA).

2.2. Animals

Female Sprague-Dawley rats were used. They were provided with water and rat chow ad libitum and housed in air-conditioned rooms that were illuminated 14 h/day. The experiments were carried out in accordance with the principles and guidelines for the use of laboratory animals and approved by the institutional research animal committee, Mansoura University, Egypt.

2.3. Theca interstitial cells isolation and culture

In order to obtain rat pre-ovulatory follicles, immature 23- to 24-day-old female rats were injected with equine chorionic gonadotropin (eCG) (10 IU) between 09:00 and 09:30 h to enhance multiple follicular development. The rats were then anaesthetized and TICs were isolated according the method described by Hoang et al. (2013). Briefly, ovaries were removed and follicles were punctured with needles to release granulosa cells (GCs) and oocysts. The remaining ovarian tissue was minced with a scalpel and digested in 100 μ L/ovary of M199 medium with 0.35 mg/mL collagenase type IA, 10 μ g/mL DNase, and 10 mg/mL BSA at 37 °C for 30 min. After digestion, the tissue was centrifuged at 1000 rpm for 4 min then the media was aspirated, and cells were resuspended in 5 mL fresh M199. Debris and oocytes were subsequently removed using 100- and 40-mm cell strainers.

Theca cell purification was performed according to the methods described by Magoffin and Erickson (1988). Discontinuous Percoll gradients were formed using both 36% and 50% percoll solutions. Theca cells were separated in the interface between the two Percoll layers and aspirated using a 20-gauge needle and syringe. The isolated cells were then washed twice in M199 and resuspended in Hepes-buffered medium 199 with 5% FCS for 24 h, followed by 24–48 h in serum-free media containing 0.1% BSA. The primary TICs were cultured in individual wells of 24-well culture plates (4 \times 105/well) (Falcon, Meylan Cedex, France), in 5% CO₂/air atmosphere and maintained at 37 °C.

3. Methods

3.1. Intracellular adenosine triphosphate (ATP) content

Intracellular ATP was measured using the Abcam assay kit according to the manufacturer's protocol. Briefly, cells (36 \times 10³) were seeded in each individual well of a 96-well plate. After overnight incubation, cells were treated with the test APs in concentrations 0.1, 1, 10 and 100 μ M for 24 h. Afterward, 50 μ L of cell lysis buffer containing an inhibitor of ATPase was added to each well, and the plate was shaken gently for 5 min. Then the 50 μ L reconstituted substrate was added to each well and the plate shaken again for another 5 min and kept in dark for 10 min before measurements. The amounts of ATP were measured with a microplate scintillation counter 'TopCount' (Perkin Elmer, Ueberlingen, Germany) enabling quantitative measurements via luminescence detected by single photon counting. Basal values in the medium were subtracted from each reading obtained in the presence of test drug then viability was expressed as a percentage from the same concentration of the vehicle control readings, presuming that the vehicle control viability is 100%.

3.2. Effect of APs on TICs oxygen consumption rates (OCRs)

TICs were incubated with the vehicle and with inhibitory concentrations 50 (IC_{50s}, defined as the concentration that inhibits 50% of the viability of control cells) and 1 μ M of the APs for 24 h. Then cells were harvested by trypsinization, centrifuged and resuspended in Hank's solution [that contained (in mM): 5.6 KCl, 138 NaCl, 4.2 NaHCO₃, 1.2 NaH₂PO₄, 2.6 CaCl₂, 1.2 MgCl₂, 10 HEPES (pH 7.4 with NaOH), and 0.1% (wt/vol) BSA) and counted by a haemocytometer. Then oxygen consumption rates (OCR) were assessed polarographically using Clark oxygen electrodes (Rank Brothers). Firstly, OCRs were studied in the basal condition for 10 min then 2 μ L of 6 mM azide solutions were added to each chamber. OCR was measured as the change in oxygen tension level over a 300-s period. In all OCR experiments; subsequent application of 6 mM azide, a blocker of cytochrome oxidase, partly

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