Review of: "Nuclear Receptor 5A2 Regulation of Agrp underlies Olanzapine-induced Hyperphagia"

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This is a multi-model approach to study Agrp regulation by a transcription factor poorly studied (NR5A2). The work of Zapata RC and colleagues aims to elucidate a possible weight gain mechanism of antipsychotic use, particularly studying this Olanzapine (OLZ) side effect in female mice. They performed several experiments both in wild type and Agrp null mice, as well as inducing a systemic inhibition and hypothalamic silencing of NR5A2. Moreover, the authors studied the possible effects of a NR5A2 commercial inhibitor (SR1848) in the hypothalamic cell line, and also the systemic administration in adult female mice.

Despite the interesting results there are some minor comments which could improve the manuscript and led the reader a better understanding of the work:

A. Concerning the methodology:

1. It should be good to clarify why the authors treated the cells with OLZ and OLZ+SR1848 for 6 hs. Also a cell viability assay could be included as supplemental material, as the work they cited did not use OLZ and was performed in a different cell line (Corzo et al, 2015).
2. About the conformation of the experimental groups: Which was the initial body weight of the different groups? Were they weight-matched? Please add this information in the text. Besides, how were animals randomized for treatment? Were the experiments performed under blinded conditions? Please state.
3. Considering that the mice are females, and that the estrous cycle influences not only the feeding behavior (Primeaux, 2011), but also the possible regulation of NR5A2 levels (Atkin et al, 2013), the authors should specify on which day the animals were sacrificed in their analysis. As well, the authors did not take into account that, throughout the experiment, hormonal levels were changing and that those variations could affect the results. Moreover, since gene expression is influenced by the circadian clock (Chun et al. 2015), it is important to describe the moment of the day at which the hypothalamus was collected.
4. The Nr5a2 siRNA experiments should be called hypothalamic silencing or knockdown of Nr5a2, because it is not a real inhibitor used there. Furthermore the concentration and amount of the siRNAs used should be included.
5. The main statistics description is missing in the materials and method section, as well as during the results description. The p values should be included and the comparisons made should be clarified, as there is some confusing information during the results description (it seems to be all individual t-tests, but it should be multiple comparisons). Besides, in some cases, the n is too small to use parametric tests, and the normal distribution of the data, and the

variance homogeneity should be evaluated (using a Shapiro-Wilk test or similar). In addition, and despite the number of samples used, it is recommended to show the individual data points rather than only the average value with the SEM. This will enable a clearer understanding of the data distribution, particularly when large error bars are displayed.

B. Concerning the results:

1. In the statement of the first result, the authors differentiate between antipsychotic-induced weight gain prone and resistant animals by presenting the body weight gain of the animals in the text (not showing any deviation error). As that data was not published before, it would help to understand if the authors add the corresponding data in the Figure 1 including the 3 groups (control, AIWG resistant and AIWG prone)
2. In Figure 1 C-D the authors present the hypothalamic mRNA relative expression of the two groups of animals mentioned. Despite the food intake and weight gain were already published (Zapata et al, 2020), the classification of prone and resistant animals as well as the mRNA levels are new. For these reasons the relative expression of control animals in both graphs should be included.
3. In Figure 2C-D it is pretty clear that the SR itself (CON+SR group) affects body weight gain and the expression of neuropeptides such as POMC and CART, why is this analysis not done?
4. Figure 2b: It is recommended to present the daily food intake as a curve, showing the daily change of it.
5. Figure 2c: Is the weight gain shown the one at the end of the dietary treatment? Please specify this in the text.
6. Interestingly, the silencing of Nr5a2 in the knockdown experiments did not reduce its expression in the KD-control animals (Figure 3A), however, it was effective in preventing OLZ-induced Nr5a2 elevations. Considering that, and the missing information regarding the concentration of the siRNA used (Materials and methods section), it should be noted that the time point analyzed was not appropriate for evaluating the silencing effect. On the other hand, the silenced OLZ animals (KD-OLZ) showed quite reduced hyperphagia, which led the reader to ask about other possible molecules involved in this behavior. In addition to these results (Fig 3), and taking into account that the authors measured Agrp in others experiments we hope to see if the quiet decreased expression or the impeded OLZ induced Nr5a2 rise affected the Agrp levels. In this way we strongly suggest including these measurements in the final version. Furthermore, all these issues concerning the silencing experiments should be included in the discussion.
7. In Figures 3C and 4C, the statistical analysis of the weight gain is missing.
8. The Agrp KO experiments showed that OLZ induced Nr5a2 raised expression was independent of Agrp levels (Figure 4), however both in culture and in wt animals OLZ induced a reduction in Cart levels (Figure 2D and 4D). The authors should include this in the discussion, as it seems to be another possible mechanism involved in OLZ hyperphagia and weight gain.
9. All the experiments performed leave to the idea that Agrp expression is under Nr5a2 control, and also the Chip assay confirms this hypothesis. In order to better understand the proposed mechanism we suggest including a diagram of the Argp promoter region analyzed in Figure 5B. Furthermore, as the authors showed a co-expression of the molecules analyzed, we strongly suggest to include a quantification of the cells. Finally, and to close this interesting work we propose to extend, if it is possible, the analysis of Figure 5 to OLZ mice.

C. Concerning the discussion:

1. It is a really interesting work, but as stated before, the discussion is too poor. Several results - like the changes in

other neuropeptides apart from Agrp in several experiments, and the results of the silencing experiments, among others - should be discussed. Furthermore, considering that the focus of this work is the upregulation of Agrp Nr5a2 mediated control induced by OLZ consumption, the authors should discuss in depth the possible mechanisms underlying this phenomena.

Bibliography

- Corzo, C. A. et al. Antiproliferation activity of a small molecule repressor of liver receptor homolog 1. Molecular pharmacology 87, 296–304, doi:10.1124/mol.114.095554 (2015).
- Primeaux, S. D. QRFP in female rats: Effects on high fat food intake and hypothalamic gene expression across the estrous cycle. Peptides, 32, 1270-1275 (2011)
- Atkin, S. D. et al. Nuclear receptor LRH-1 induces the reproductive neuropeptide kisspeptin in the hypothalamus. Molecular endocrinology 27, 598–605, doi:10.1210/me.2012-1371 (2013).
- Chun, L. E. et al. Variations in Phase and Amplitude of Rhythmic Clock Gene Expression across Prefrontal Cortex, Hippocampus, Amygdala, and Hypothalamic Paraventricular and Suprachiasmatic Nuclei of Male and Female Rats. Journal of Biological Rhythms 30, 417-436. doi:10.1177/0748730415598608 (2015)
- Zapata, R. C. & Osborn, O. Susceptibility of male wild type mouse strains to antipsychotic-induced weight gain. Physiology & behavior 220, 112859, doi:10.1016/j.physbeh.2020.112859 (2020).