5-HT2A deletion protects against Clozapine-induced hyperglycemia

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ABSTRACT
Clozapine is an antipsychotic known for its superior efficacy in treating drug-resistant Schizophrenia. However, Clozapine induces various side effects such as hyperglycemia, agranulocytosis, weight gain etc. The mechanisms of these Clozapine-induced side effects have remained largely elusive though an important role is ascribed to 5-HT2A (Serotonin receptor subtype-2A). In this pilot study, we report for the first time that the 5-HT2A ‘global’ knockout mice (Htr2a+/−) are resistant to the Clozapine-induced hyperglycemia. Importantly though, the Htr2a+/− mice exhibit near normal basal glucose metabolism in the glucose tolerance tests. Collectively, the Htr2a+/− mice provide an important tool to study the Clozapine-induced hyperglycemia.

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Among atypical antipsychotics, Clozapine is considered to be the most effective, clinically. However, it is associated with the increased risk of metabolic disorders such as weight gain and glucose dysregulation.1–4

Clozapine acts as an inverse agonist/antagonist at the Serotonin receptor 5-HT2A, blocking the canonical signaling.5 However, recently Clozapine was shown to have agonist-like properties at the 5-HT2A.6,7 5-HT2A knockout mice (Htr2a+/−) are also resistant to a side effect of Clozapine, i.e. Sedation.8,9 Here we have studied the response of the Htr2a+/− mice to Clozapine-induced metabolic side effects, particularly the acute Clozapine-induced hyperglycemia (CIH). We have used global Htr2a+/− mice, where 5-HT2A is absent from all tissues from fertilization.

CIH is observed in patients and also in animal models.2–4 CIH is reversible and ceases with the treatment.4 Therefore it would be very useful to study the acute form of CIH, disentangled from the chronic side effects such as weight gain. The mechanism of CIH with respect to its receptor dependence, if any, has not been well studied. 5-HT2A is one of the potential candidates for CIH.

5-HT2A is expressed in the brain10 and in several peripheral organs.11 5-HT2A agonists have been shown to increase glucose uptake, which is inhibited by the 5-HT2C class antagonist- Ketanserin.11 Moreover, Ketanserin impairs insulin sensitivity in healthy volunteers.12 Conversely, 5-HT2A agonists have also been shown to cause hyperglycemia in animals.13 Since these ligands can have multiple targets and distinct pharmacokinetics, the role of 5-HT2A in CIH is hard to discern. Therefore, we used the Htr2a+/− mice to address this question.6

The Htr2a+/− strain was maintained under standard laboratory conditions.7 Male mice, minimum 3 months old, were used for the experiments. The mice were obtained from heterozygous matings and genotyped as described in Joshi et al., 2016.8 Mice were randomly assigned to either the vehicle or the drug group for CIH. All experiments were approved by the Institutional Animal Ethics Committee (NCBS-IAE-2016/15(E)).

For CIH we arrived at a dose of 5 mg/kg of Clozapine based on the following a) at this dose our group and others have reported differences between Htr2a+/− and Htr2a+/− mice for Clozapine-induced sedation b) at 5 mg/kg of Clozapine we have observed 5-HT2A dependent Clozapine-specific cellular responses in the mice brain (Joshi et al., BioRxiv 226050) c) and previous literature.14 For the CIH test, mice were not allowed to feed for 6 hours prior to the drug administration to avoid immediate effects of feeding on the blood glucose levels (BGL) (Fig. 1a). Clozapine (0444, Tocris, Bristol, UK) was administered intraperitoneally, and blood was obtained at the defined intervals from the tail tip. The BGL were determined with
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of CIH. It would be of interest to determine if the observed effects in the Htr2a<sup>−/−</sup> mice are a result of a direct interaction of Clozapine with the 5-HT<sub>2A</sub> or a secondary effect. It would also be vital to determine if any compensatory changes in the expression of other GPCRs (such as 5-HT<sub>1A</sub>, 5-HT<sub>2B</sub>, 5-HT<sub>2C</sub>, and H1 receptor etc.) and the endocrine system are present in the Htr2a<sup>−/−</sup> mice. Since we have used a global knockout of the 5-HT<sub>2A</sub> we cannot dissect the roles played by specific tissues in CIH. Genetic or virus-induced tissue-specific deletion of 5-HT<sub>2A</sub> would be appropriate in this regard. While this study has explored very specific, and a limited aspect of the metabolic side effects of Clozapine, namely hyperglycemia, it should still serve as a stepping stone to understand the chronic metabolic side effects of Clozapine.

Contributors

Author RJ, SPS performed experiments. All authors contributed to the design of the study and have approved the final manuscript.

Conflict of interest

The authors declare no conflict of interest.

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