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Short Communication

5-HT_{2A} 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-HT_{2A} (Serotonin receptor subtype-2A). In this pilot study, we report for the first time that the 5-HT_{2A} '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.

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-HT_{2A}, blocking the canonical signaling.[5] However, recently Clozapine was shown to have agonist-like properties at the 5-HT_{2A}.[6,7] 5-HT_{2A} 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-HT_{2A} 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-HT_{2A} is one of the potential candidates for CIH.

5-HT_{2A} is expressed in the brain[10] and in several peripheral organs.[11] 5-HT_{2A} agonists have been shown to increase glucose uptake, which is inhibited by the 5-HT_2 class antagonist- Ketanserin.[11] Moreover, Ketanserin impairs insulin sensitivity in healthy volunteers.[12] Conversely, 5-HT_{2A} 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-HT_{2A} in CIH is hard to discern. Therefore, we used the *Htr2a*$^{-/-}$ mice to address this question.[9]

The *Htr2a*$^{-/-}$ strain was maintained under standard laboratory conditions.[9] 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.[9] 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-HT_{2A} 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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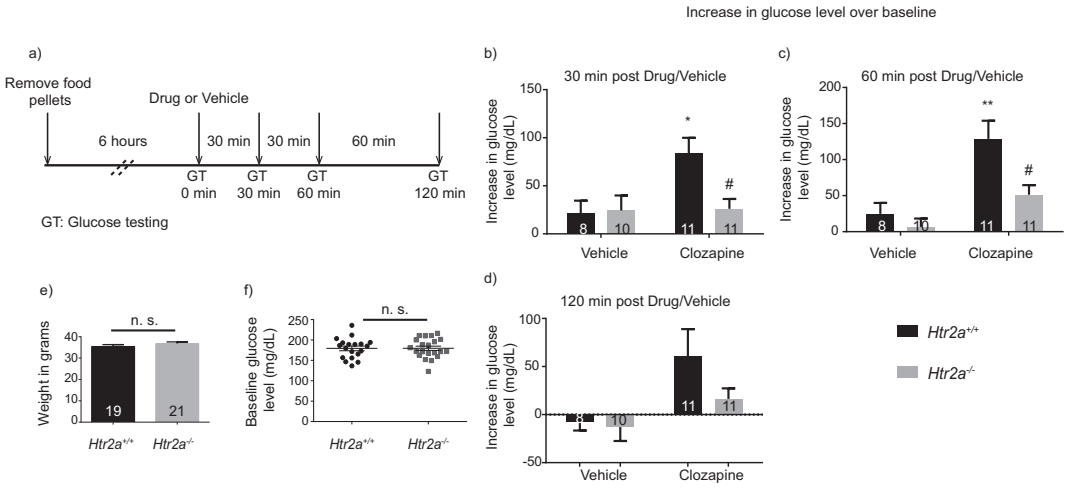

Fig. 1. *Htr2a*$^{-/-}$ mice are protected against the CIH. a) Schematic representation of the protocol for CIH. b), c) and d) The graphs show Clozapine-induced increase in the BGL- 30, 60 and 120 min after the drug/vehicle administration. The *Htr2a*$^{+/+}$ mice showed a significant increase in the BGL compared to the vehicle-treated group and the *Htr2a*$^{-/-}$ group at 30 and 60 min time points. After 120 min of drug administration, the *Htr2a*$^{+/+}$ mice only showed a trend towards increased BGL, which did not reach significance. two-way ANOVA – *Comparison with 0 mg/kg, # comparison with the *Htr2a*$^{+/+}$ at the same dose. e & f) Basal body weight and BGL did not differ between the *Htr2a*$^{+/+}$ and *Htr2a*$^{-/-}$ mice. Student's t-test. (n. s. – not significant). Number in the bar represents 'N's. Data shown as Mean ± SEM. *$p < 0.05$, **$p < 0.001$. (two-way ANOVA, 30 min post-drug treatment: effect of drug, $F(1,36) = 4.87$, $p = 0.0337$, genotype, $F(1,36) = 3.62$, $p = 0.0648$, interaction, $F(1,36) = 4.34$, $p = 0.0444$, 60 min post-drug treatment: effect of drug, $F(1,36) = 17.04$, $p = 0.0002$, genotype, $F(1,36) = 7.06$, $p = 0.0116$, interaction, $F(1,36) = 2.72$, $p = 0.1077$).

'Glucose One Touch Ultra 2 meter' (Johnson and Johnson, New Jersey, USA). At least two readings were acquired for each time point.

After 30 and 60 minutes of Clozapine administration, the *Htr2a*$^{+/+}$ group showed a drastic increase in the BGL compared to the vehicle group, indicating CIH (Fig. 1b,c). However, the *Htr2a*$^{-/-}$ mice showed very little or no increase in the BGL. The increase in the BGL in the *Htr2a*$^{+/+}$ group was significantly higher than the *Htr2a*$^{-/-}$ group (Fig. 1b,c). At 120 minutes post-drug administration, the BGL in most of the Clozapine-treated *Htr2a*$^{+/+}$ group approached basal levels. Thus, at 120 minutes, the *Htr2a*$^{+/+}$ mice exhibited a trend of CIH compared to the vehicle and the *Htr2a*$^{-/-}$ group (Fig. 1d). Therefore our results suggest that the lack of 5-HT_{2A} modulates CIH.

Importantly, our experiments were conducted with littermates and the baseline body weights and BGL of the *Htr2a*$^{+/+}$ and *Htr2a*$^{-/-}$ mice were comparable to each other (Fig. 1e,f). These baseline measurements were determined just before the administration of Clozapine/Vehicle, and the data was pooled from all the *Htr2a*$^{+/+}$ and *Htr2a*$^{-/-}$ mice used.

To account for any pre-existing differences in the glucose metabolism of the *Htr2a*$^{+/+}$ and *Htr2a*$^{-/-}$ mice, we conducted glucose tolerance tests (GTT). For GTT, mice were food deprived overnight, and blood samples were obtained as described above. To reduce the number of animals, mice were subjected to the GTT, followed by the CIH test, with a minimum interval of a one-week. Glucose (1 mg/kg) (G8270, Sigma–Aldrich, USA) was administered intraperitoneally.

Glucose administration produced the expected and similar patterns of the BGL in both the *Htr2a*$^{+/+}$ and *Htr2a*$^{-/-}$ mice (Fig. 2a). We further analyzed the total area under the curve (AUC). However, it was statistically indistinguishable between the *Htr2a*$^{+/+}$ and *Htr2a*$^{-/-}$ mice (Fig. 2b). These results suggest that the basal glucose metabolism in the *Htr2a*$^{-/-}$ and *Htr2a*$^{+/+}$ mice is largely similar and unlikely to explain the differences seen with CIH.

Interestingly, there was a subtle yet significant difference between the baseline BGL of the *Htr2a*$^{+/+}$ and *Htr2a*$^{-/-}$ mice (94.89 (±3.8) and 120.4 (±7.5) mg/dL, respectively), after 12 h of food deprivation (Fig. 2a). 5-HT_{2A} expression in the liver and muscles is thought to regulate glucose metabolism.[11,15] Thus, it is possible that the lack of 5-HT_{2A} exerts subtle effects on glucose metabolism which builds up over prolonged fasting. Further experiments on the basal metabolism of the *Htr2a*$^{-/-}$ mice under different dietary regime might shed some light on this aspect.

Taken together this report presents the *Htr2a*$^{-/-}$ mice as a useful tool to investigate some of the pathways underlying the acute form

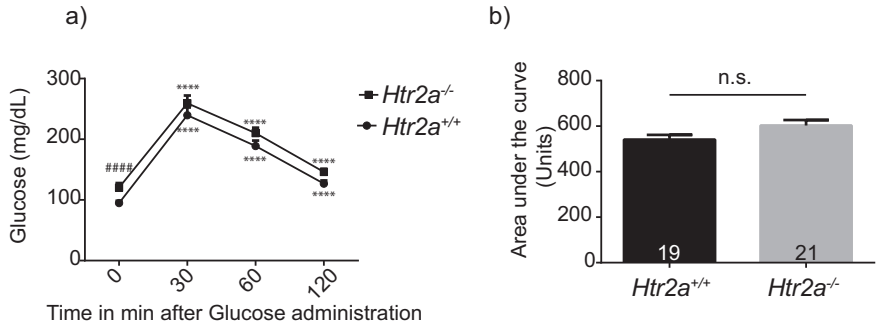

Fig. 2. *Htr2a*$^{+/+}$ and *Htr2a*$^{-/-}$ mice show similar basal glucose metabolism. a) The *Htr2a*$^{+/+}$ and *Htr2a*$^{-/-}$ mice showed similar and a clear increase in the BGL, 30 min after glucose administration. two-way ANOVA – *Comparison with 0 mg/kg, # comparison with *Htr2a*$^{+/+}$ at the same dose. (Data was transformed for two-way ANOVA, effect of time, $F(3,114) = 217.9$, $p < 0.0001$, genotype, $F(1,38) = 9.147$, $p = 0.0044$, interaction, $F(3,114) = 4.501$, $p = 0.0051$). b) The *Htr2a*$^{+/+}$ and *Htr2a*$^{-/-}$ mice showed no difference in the Area Under the Curve in Figure 2a (AUC). Student's t-test. (n. s. – not significant). Number in the bar represents 'N's. Data shown as Mean ± SEM. ****$p < 0.0001$.

of CIH. It would be of interest to determine if the observed effects in the *Htr2a*$^{-/-}$ mice are a result of a direct interaction of Clozapine with the 5-HT$_{2A}$ 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$_{1A}$, 5-HT$_{2B}$, 5-HT$_{2C}$, and H1 receptor etc.) and the endocrine system are present in the *Htr2a*$^{-/-}$ mice.

Since we have used a global knockoutof the 5-HT$_{2A,}$ we cannot dissect the roles played by specific tissues in CIH. Genetic or virus-induced tissue-specific deletion of 5-HT$_{2A}$ 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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