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5-HT_{2C}Rs Expressed by Pro-opiomelanocortin Neurons Regulate Insulin Sensitivity in Liver

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Abstract

Mice lacking 5-HT 2C receptors (5-HT_{2C}Rs) displayed insulin resistance in the liver, a phenotype normalized by re-expression of 5-HT_{2C}Rs only in pro-opiomelanocortin (POMC) neurons. 5-HT_{2C}R deficiency also abolished anti-diabetic effects of mCPP (a 5-HT_{2C}R agonist) while such effects were restored in mice with 5-HT_{2C}Rs re-expressed in POMC neurons. Our findings demonstrated that 5-HT_{2C}Rs expressed by POMC neurons are physiologically relevant regulators of insulin sensitivity and glucose homeostasis in the liver.

5-hydroxytryptamine 2C receptors (5-HT_{2C}Rs) in the brain regulate energy balance and glucose homeostasis1. A selective 5-HT_{2C}R agonist, metachlorophenylpiperazine (mCPP), ameliorates insulin resistance and glucose intolerance in mice with diet-induced obesity (DIO)2. However, mechanisms underlying these effects are unclear. Pro-opiomelanocortin (POMC) neurons in the hypothalamic arcuate nucleus (ARC) produce α-melanocyte-stimulating hormone (α-MSH), an agonist of melanocortin 4 receptors (MC4Rs)3. The central melanocortin system plays essential roles in the regulation of feeding and glucose homeostasis3. POMC neurons express 5-HT_{2C}Rs4 and receive innervation from serotoninergic neurons5. 5-HT drugs, including mCPP, activate POMC neurons4. In

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Author Contribution YX conducted glucose/insulin experiments and wrote the manuscript; EDB performed clamp studies; JWS and KWW conducted electrophysiological recordings; JJC, MF and JR contributed to the islet studies; JEJ, JMZ and BBL generated mice; WHL, PES and BBL assisted in data intepreation; JKE supervised the project.

addition, 5-HT_{2C}R agonists stimulate POMC expression2. Using mice with global 5-HT_{2C}R deficiency (*2C null* mice) and mice with 5-HT_{2C}Rs expressed only in POMC neurons (*2C/POMC* mice), we have demonstrated that POMC neurons contribute to the ability of 5-HT_{2C}R agonists to suppress feeding6. Here we sought to use the same mouse models to determine whether 5-HT_{2C}Rs expressed by POMC neurons also mediate 5-HT_{2C}Rs' effects on glycemic control.

As reported previously6, *2C null* mice do not express 5-HT_{2C}Rs. In 2C/POMC mice, 5-HT_{2C}Rs are re-expressed only in brain sites where POMC is expressed (the hypothalamus and brainstem). Further, using electrophysiological recordings, we found that mCPP depolarized 26% POMC neurons in *WT* mice (Fig. 1). This mCPP-induced depolarization was abolished in POMC neurons from *2C null* mice, but restored in *2C/POMC* mice (Fig. 1). Therefore, 5-HT_{2C}Rs are selectively re-expressed in POMC neurons in *2C/POMC* mice. It should be noted that POMC is also expressed by neurons in the nucleus of solitary tract (NTS)7. Thus we cannot rule out that the small number of POMC neurons in the NTS may contribute to the responses outlined below.

Since 2C null mice display late-onset obesity and hyperadiposity6, phenotypes that may interfere with glucose homeostasis, all studies were performed in young mice with matched body weight and/or body adiposity (Supplemental Table 1). We first assessed the insulin sensitivity using insulin tolerance tests (ITTs). While intraperitoneal injections of insulin induced robust decreases in blood glucose in chow-fed *wildtype* (*WT*) mice, *2C null* littermates were significantly resistant to insulin (Fig. 2a). Notably, insulin sensitivity in *2C/POMC* mice was rescued to *WT* level (Fig. 2a), indicating that 5-HT_{2C}Rs expressed by POMC neurons were sufficient to normalize insulin resistance resulting from global 5-HT_{2C}R deficiency. Despite insulin resistance, *2C null* mice showed normal glucose clearance rate in glucose tolerance tests (GTTs) (Fig. 2b), and normal glucose levels at fed and fasted conditions (Fig. 2c). The basal insulin levels at fed condition were slightly but not significantly higher in *2C null* mice than in *WT* and *2C/POMC* littermates (Figure 2d). Fasted insulin levels were comparable in these mice (Figure 2d). Similar phenotypes were observed in mice fed with high fat diet (HFD) (Supplemental Fig. 1a–d). Since *2C null* mice had normal glucose clearance rate while being insulin resistant, islets in these mice may have undergone compensatory adaptations which allowed elevated insulin secretion in response to hyperglycemia. Supporting this hypothesis, we found that a glucose load (0.75g/kg, i.p.) induced a significantly elevated serum insulin level in *2C null* mice than in *WT* mice, and this hyperinsulinemia was normalized in *2C/POMC* mice (Fig. 2e). Consistently, we found that *in vitro* glucose-stimulated insulin secretion was significantly potentiated in *2C null* islets when compared to islets isolated from *WT* and *2C/POMC* mice (Supplemental Figure 1e). We cannot rule out the possibility that the increased islet sensitivity to glucose challenge may be the direct outcome of 5-HT_{2C}R deficiency. However, it is more likely that this islet phenotype in *2C null* mice is a secondary adaptation which occurs to compensate for insulin resistance.

We next performed the hyperinsulinemic-euglycemic clamp studies to assess mechanisms underlying the insulin resistance found in ITT studies. We found the glucose infusion rate (GIR) needed to maintain eugylcemia was reduced in *2C null* mice compared to *WT*

littermates (Figure 2f). GIR in *2C/POMC* mice was fully restored to *WT* levels (Fig. 2f). In addition, the basal hepatic glucose production (HGP) and glucose disposal were higher in *2C null* versus *WT* mice (Fig. 2g–h). The expected insulin-mediated suppression of HGP and increase in glucose disposal were evident in *WT* mice (Fig. 2g–h). A comparable rise in insulin in *2C null* mice during the clamp failed to fully suppress HGP whereas glucose disposal rates were comparable (Fig. 2g–h). Notably, insulin-mediated suppression of HGP was fully restored in *2C/POMC* mice (Fig. 2g). Collectively, these results demonstrated that insulin resistance caused by global 5-HT_{2C}R deficiency was due to impaired hepatic insulin action and re-expression of 5-HT_{2C}Rs only in POMC neurons was sufficient to rescue this impairment. These findings support the model that 5-HT_{2C}R-melanocortin circuits regulate the liver to control peripheral insulin sensitivity and glucose balance.

To further investigate whether 5-HT_{2C}Rs expressed by POMC neurons are sufficient to mediate effects of 5-HT drugs on glucose homeostasis, we tested the effects of mCPP on GTTs and ITTs in DIO mice. Pre-treatments with mCPP (1.5 mg/kg, i.p.) in *WT* DIO mice significantly improved glucose tolerance and insulin sensitivity (Fig. 2i–j). In contrast, mCPP failed to produce these anti-diabetic effects in *2C null* mice (Fig. 2i–j). Remarkably, mCPP-induced improvement in the GTTs and ITTs were restored in *2C/POMC* mice (Fig. 2i–j). Therefore, our results support the model that 5-HT_{2C}Rs expressed by POMC neurons are sufficient to mediate the anti-diabetic effects of 5-HT_{2C}R agonists.

Activation of 5-HT_{2C}Rs in POMC neurons has been shown to stimulate firing activity of these neurons (see reference 4 and Fig. 1). This presumably leads to increased release of neurotransmitters (e.g. α-MSH, glutamate, etc.), which may underlie the mechanisms by which 5-HT_{2C}R regulates glucose homeostasis. In addition, 5-HT_{2C}R agonists may also regulate production of neurotransmitters in POMC neurons. Supporting this notion, 5-HT_{2C}R agonists stimulate POMC expression2. Consistent with this observation, we found that *2C null* mice had significantly lower POMC expression in the ARC compared to their *WT* littermates, a phenotype that was restored in *2C/POMC* mice (Supplemental Figure 1f).

It is important to note that our results do not exclude the possibility that redundant 5-HT_{2C}R populations may also mediate similar effects. For example, 5-HT_{2C}Rs are found in other CNS regions implicated in the regulation of glucose homeostasis, including the paraventricular nucleus of the hypothalamus, the ventromedial hypothalamic nucleus, and other sites8. These regions all receive serotoninergic projections9. The physiological relevance of 5-HT_{2C}Rs in these sites is yet to be characterized. However, our unique mouse model will allow us to directly address the importance of 5-HT_{2C}Rs in any site in which specific expression of Cre-recombinase can be directed. In conclusion, we have provided evidence that 5-HT_{2C}Rs expressed by POMC neurons are physiologically important in regulating hepatic glucose production and insulin sensitivity. Moreover, this 5-HT_{2C}R-melanocortin circuit is sufficient to mediate the anti-diabetic effects of 5-HT_{2C}R agonists.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgment

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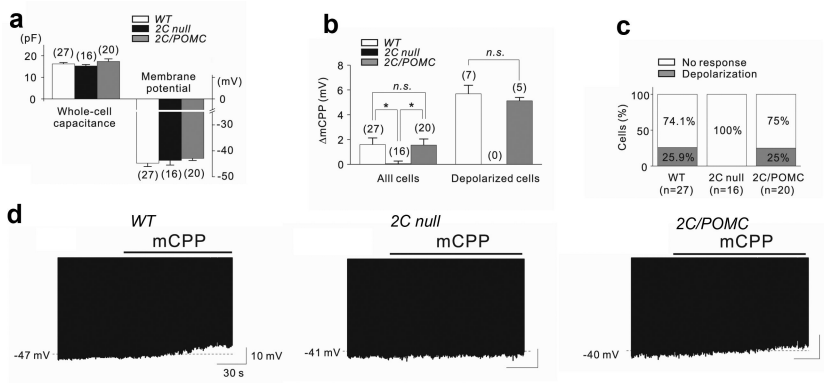

Figure 1.

mCPP-induced depolarization of POMC neurons is abolished in *2C null* mice, but restored in *2C/POMC* mice. (a) Whole-cell capacitance and resting membrane potential of POMC neurons. The numbers of cells recorded are indicated in the parentheses. (b) mCPP-induced changes in membrane potential (ΔmCPP) from all recorded POMC neurons (left bars) and depolarized POMC neurons only (right bars). *, P<0.05. (c) The percentage of POMC neurons that depolarized in response to mCPP. (d) Representative membrane potential traces. Horizontal bars indicate the period of mCPP treatment. Dotted lines indicate the resting membrane potential. Scale bars indicate 10 mV (vertical) and 30 s (horizontal). All data are presented as mean ± s.e.m.

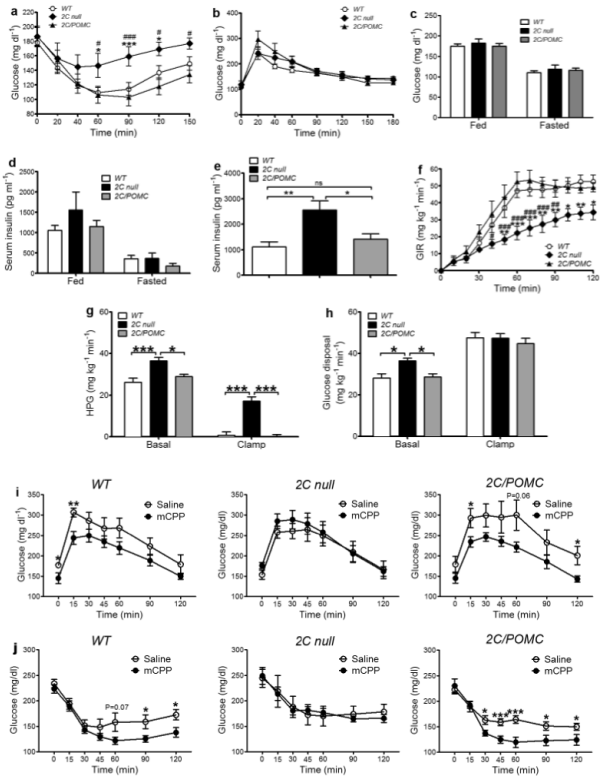

Figure 2.

Re-expression of 5-HT_{2C}Rs in POMC neurons rescues insulin resistance. (a) Insulin tolerance tests in chow-fed mice (insulin 1 U/kg). N=8 or 9/genotype. * or ***, P<0.05 or P<0.001 between *2C null* vs *WT* mice; # or ###, P<0.05 or P<0.001 between *2C null* vs *2C/POMC* mice. (b) Glucose tolerance tests in chow-fed mice (glucose 1 g/kg). N=5–12/genotype. (c) Serum glucose at fed or fasted conditions in chow-fed mice. N=6–12/genotype. (d) Serum insulin at fed or fasted conditions in chow-fed mice. N=12–23/genotype. (e) Serum insulin at 30 min after HFD-fed mice received a glucose bolus injection (0.75 g/kg). N=6–14/genotype. * or **, P<0.05 or P<0.01. (f) Glucose infusion rate during a hyperinsulinemic-euglycemic clamp in chow-fed mice. N=7–9/genotype. *, ** or ***, P<0.05, P<0.01 or P<0.001 between *2C null* vs *WT* mice; #, ## or ###, P<0.05, P<0.01 or P<0.001 between *2C null* vs *2C/POMC* mice. (g) Hepatic glucose production at basal (pre-clamp) or hyperinsulinemic clamp conditions. N=7–9/genotype. * or ***, P<0.05 or P<0.001. (h) Glucose disposal rate at basal (pre-clamp) or hyperinsulinemic clamp conditions. N=7–9/genotype. *, P<0.05. (i) Intraperitoneal injections of saline or mCPP (1.5 mg/kg), followed by GTT (0.75 g/kg) in *WT*, *2C null* and *2C/POM* mice. (j) Intraperitoneal injections of saline or mCPP (1.5 mg/kg), followed by ITT (1.5 U/kg) in *WT*, *2C null* and *2C/POM* mice. N=8–9/group. *, ** or ***, P<0.05, P<0.01 or P<0.001 between saline vs mCPP. All data are presented as mean ± s.e.m. Care of all animals and procedures were approved by UTSW Institutional Animal Care and Use Committees.