2018

Decreased 5-HT2cR and GHSR1a interaction in antipsychotic drug-induced obesity

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Publication Details

Huang, X., Weston-Green, K. & Yu, Y. (2018). Decreased 5-HT2cR and GHSR1a interaction in antipsychotic drug-induced obesity. Obesity Reviews, 19 (3), 396-405.

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Abstract

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Disciplines

Medicine and Health Sciences

Publication Details

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Review:

Decreased 5-HT2cR and GHSR1a interaction in antipsychotic drug-induced obesity

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Running title: 5-HT2cR and GHSR1a interaction in antipsychotic drug-induced obesity

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Abstract:

Second generation antipsychotics (SGAs), notably atypical antipsychotics including olanzapine, clozapine and risperidone, can cause weight gain and obesity side effects. Antagonism of serotonin 2c receptors (5-HT2cR) and activation of ghrelin receptor type 1a (GHSR1a) signalling have been identified as a main cause of SGA induced obesity. Here we review the pivotal regulatory role of the 5-HT2cR in ghrelin-mediated appetite signalling. The 5-HT2cR dimerizes with GHSR1a to inhibit orexigenic signalling, while 5-HT2cR antagonism reduces dimerization and increases GHSR1a-induced food intake. Dimerization is specific to the unedited 5-HT2cR isoform. 5-HT2cR antagonism by SGAs may disrupt the normal inhibitory tone on the GHSR1a, increasing orexigenic signalling. The 5-HT2cR and its interaction with the GHSR1a could serve as the basis for discovering novel approaches to preventing and treating SGA-induced obesity.

Keywords: serotonin 2c receptors (5-HT2cR), ghrelin receptor type 1a (GHSR1a), heterodimer, antipsychotic drug-induced obesity

1. Atypical antipsychotic-induced obesity

Antipsychotic medication is commonly used in the clinic to treat schizophrenia, bipolar disorder and other psychotic disorders. Despite more than a decade of scientific research, the side-effect of obesity caused by current second generation antipsychotics (SGAs) remains unresolved. Indeed, the prevalence of weight gain side-effects caused by SGAs range from 42 – 64% of treated patients [1-3]. In long-term (≥48 weeks) studies of olanzapine, the mean weight gain is 5.6 kg [1]. Understandably, weight gain side-effects are strong predictors of medication non-compliance, which is a primary barrier to the effective treatment of these serious psychiatric illnesses [4, 5]. Medication non-compliance is particularly problematic due to the astounding 5-fold increased risk of symptom relapse, hospitalisation and negative long-term outcomes [6, 7]. In addition, the relationship between obesity and antipsychotic drug use has been reported in the adult population through a longitudinal, retrospective claim database study [8]. The Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE) trial revealed average body mass indexes (kg/m^2) of 33.0±8.1 in female and 28.5 ± 6.2 in male schizophrenia patients with a history of chronic antipsychotic drug treatment [3]. Furthermore, the prevalence of metabolic syndrome is reportedly 52% and 36% of female and male schizophrenia patients, respectively [3]. Obesity and metabolic syndrome are serious risk factors for further chronic illnesses, such as dyslipidaemia, type 2 diabetes mellitus, cardiovascular disease and stroke, which are major global health concerns. These health issues highlight the urgency of understanding the mechanisms underlying SGA-induced body weight gain and obesity.

SGA-induced obesity is caused by over-eating, decreased energy expenditure and altered energy metabolism [9-11]. For example, a randomized double-blind study reported increased food craving with olanzapine and clozapine treatment [9], and a low resting energy expenditure was identified in SGA-treated male patients [10]. Furthermore, in female animal studies, olanzapine increases food intake and reduces temperature and thermogenesis in brown adipose tissue (BAT) [12, 13]. Numerous factors in the brain and peripheral tissues that regulate appetite, metabolism and body

weight can be dysregulated by SGA treatment. SGAs directly interact with a range of neurotransmitter receptors that are involved in energy homeostasis, such as 5-HT subtypes 2C (5HT-2cR) and 2A, histaminergic H1 receptor, adrenergic α, muscarinic M3 and dopaminergic D2 receptors. In clinical and rodent studies, SGAs increase circulating ghrelin levels [14]. Moreover, rats treated with olanzapine have significantly elevated expression of the hypothalamic ghrelin receptor (also called growth hormone secretagogue receptor 1a; GHS-R1a) [14, 15]. There is a growing body of evidence to suggest a key regulatory role for the serotonin 5-HT2cR in GHSR1a signalling. It was recently identified that the 5-HT2cR interacts with the GHSR1a, forming a heterodimer that inhibits ghrelin signalling [16, 17]. This review explores the role of central 5-HT2cR antagonism and GHSR1a molecular pathways in SGA-induced obesity.

2. Hypothalamic Ghrelin-GHSR1a-NPY/AgRP Pathway in Body Weight Control and Obesity

2.1 Ghrelin-GHSR1a-NPY/AgRP Pathway: Ghrelin is primarily produced by endocrine cells (X/A-like cells) in the gastric mucosa of humans and rodents [18]. It is the only known peripheral orexigenic hormone that increases food intake and body weight through actions on the hypothalamus. Central administration of ghrelin increases food intake, up-regulates lipogenic enzyme expression in adipocytes, and decreases thermogenesis-related mitochondrial uncoupling proteins in brown adipose tissue through activation of GHSR1a pathways in the hypothalamus [19, 20]. For example, the intracerebroventricular (i.c.v.) administration of ghrelin rapidly increases feeding and this orexigenic effect is sustained for 24 hours in rats [19]. Ghrelin (i.c.v.) markedly up-regulates lipogenic enzyme expression and mRNA levels of the fat storage–promoting enzymes lipoprotein lipase (LPL), acetyl-CoA carboxylase α (ACC), fatty acid synthase (FAS), and stearoyl-CoA desaturase–1 (SCD1) in white adipose tissue (WAT) of rats [20]. Ghrelin (i.c.v) also markedly decreases the expression of thermogenesis-related mitochondrial uncoupling protein 1 (UCP1) in BAT of rats during both *ad libitum* and pair-feeding dietary paradigms [20]. Concerning the

mechanisms by which central ghrelin infusion modulates peripheral adipocyte metabolism, several lines of evidence suggest a role for the autonomic nervous system, as WAT and BAT are mostly innervated by the sympathetic nervous system [21, 22], and ghrelin-induced changes in adipocyte metabolism fail to occur in TKO (triple β1-, β2-, and β3-adrenoceptor knockout) mice lacking sympathetic nervous system signalling [20].

Endogenous ghrelin targets GHSR1a in the hypothalamic arcuate nucleus (Arc) through the blood brain barrier of the adjacent medial eminence [23]. In the Arc, more than 90% neuropeptide Y (NPY)/agouti-related peptide (AgRP) neurons express GHSR1a; however, only 8% pro-opiomelanocortin (POMC) neurons express GHSR1a [24]. Activation of the GHSR1a stimulates food intake and fat deposition primarily through intracellular signalling pathways that increase orexigenic NPY and AgRP, and suppress anorexigenic POMC signalling in the hypothalamus [23, 25, 26]. Therefore, there is an important regulatory role for the hypothalamic GHSR1a in metabolic homeostasis through its effects on orexigenic NPY/AgRP, appetite and body weight. Interestingly, olanzapine upregulates GHSR1a, NPY and AgRP in the Arc, suggesting that GHSR1 signaling in hypothalamic NPY and AgRP neurons are involved in the SGA-induced elevation of food intake [27].

2.2 The GHSR1a Intracellular Signalling Pathway (GHSR1a-AMPK-NPY/AgRP): The GHSR1a stimulates several signalling pathways [14]; however, research has highlighted an important role for the 5' AMP-activated protein kinase (AMPK) pathway in ghrelin-induced food intake via NPY and AgRP up-regulation. For example, ghrelin increases hypothalamic AMPK in rodents *in-vivo* and in NPY neurons *in-vitro*, GHSR1a knock-out mice do not exhibit ghrelin-induced hypothalamic AMPK activation or hyperphagia, while AMPK inhibition decreases ghrelin-stimulated food intake [26]. The intracellular signalling pathway stimulated by the GHSR1a and AMPK is shown in Figure 1. Briefly, GHSR1a activation promotes mitochondrial β-oxidation through AMPK phosphorylation. AMPK activates carnitine palmitoyl transferase 1 (CPT1), which

transports fatty acids into the mitochondria. Inhibition of CPT1 prevents ghrelin-induced NPY and AgRP up-regulation [26]. Mitochondrial fatty acid oxidation stimulates uncoupling protein-2 (UCP2) activity, which is an important contributor to the energy capacity of the neuron. GHSR1a-induced activation of the AMPK-CPT1-UCP2 pathway increases AgRP and NPY mRNA expression by modulating intracellular transcriptional factors, forkhead box O1 (FOXO1) and the phosphorylated cAMP-response element-binding protein (pCREB), which are translocated to the nucleus to initiate NPY and AgRP promoter activity, respectively [28-30]. Finally, the transcription factor, brain-specific homeobox (BSX), interacts with FOXO1 and pCREB to enhance NPY and AgRP expression [31]. In addition, the transcription factor FOXO1 also suppresses POMC expression, i.e.: five residues on FOXO1, Gln145, Arg147, Lys148, Arg153 and Arg154, are necessary to inhibit POMC promoter activity [32]. However, given the low abundance of GSHR1a on POMC neurons it is unlikely that direct effects of GHSR1a signalling on POMC neurons plays a significant role in appetite control [26]. On the other hand, suppression of anorexigenic POMC neurons through inhibitory GABA interactions from NPY and AgRP neurons has potent orexigenic effects [33]. Overall, GHSR1a stimulates the AMPK-CPT1-UCP2 axis to initiate gene expression of NPY and AgRP, which suppress anorexigenic POMC neurons through inhibitory GABA interactions to stimulate appetite [26, 33] (Figure 2).

2.3 Hypothalamic Circuits Regulating Body Weight: Hypothalamic NPY, AgRP and POMC neurons of the Arc send projections to second order neurons in several key regions: the paraventricular nucleus of hypothalamus (PVN), ventromedial nucleus of the hypothalamus (VMH), dorsomedial hypothalamic nucleus (DMH) and lateral hypothalamus (LH) [34, 35] (Figure 2). For example, the PVN expresses high levels of melanocortin receptor sub-types, 3 and 4 (MC3R and MC4R) as well as NPY Y1 and Y5 receptors that are involved in appetite regulation by the diverse afferent inputs (eg: from NPY/AgRP and POMC neurons of the Arc) [36]. The thyrotropin-releasing hormone (TRH) and corticotrophin-releasing hormone (CRH) expressed in the PVN are also involved in the control of energy balance [37-39]. Elevated Arc NPY expression leads to a

marked reduction in tyrosine hydroxylase (TH) mRNA and protein expression in the PVN, an effect that is mediated by Y1 receptors [40]. In the VMH, steroidogenic factor 1 (SF1) and brain-derived neurotrophic factor (BDNF) play significant roles in the control of energy balance [41-43]. A number of neuropeptides involved in the control of appetite and energy balance (such as NPY and CRH) are expressed within the DMH [44]. For example, there is a high level of NPY expression in the DMH of mice fed a high-fat diet, but this expression is not evident during a normal lab chow diet [45]. The LH is another region that receives inputs from Arc NPY/AgRP and POMC neurons, mediating orexigenic responses through orexin and melanin-concentrating hormone (MCH) [45]. In addition to the hypothalamic nuclei described above, Arc NPY/AgRP and POMC neurons project to extrahypothalamic areas, including discrete regions of the brainstem (such as the nucleus tractus solitaries (NTS) via sympathetic noradrenergic (A1 area) and adrenergic (C1 area) innervation, as well as the parabrachial nucleus (PBN)) to regulate energy intake, BAT thermogenesis, and WAT lipolysis and lipogenesis [35]. For example, inhibitory GABAergic signalling from NPY/AgRP neurons to the PBN is crucial in the control of feeding responses [46]. One study reported that acute ablation of NPY/AgRP/GABA neurons in adult mice using diphtheria toxin (DT) leads to severe anorexia [47]. Indeed, inactivation of GABA biosynthesis in the Arc or blockade of GABA receptors in the PBN of mice promotes anorexia [47]. Overall, hypothalamic NPY, AgRP and POMC neurons induce wide-spread effects on appetite signalling in the brain and metabolic homeostasis. Therefore, alterations to upstream factors that influence these signals, such as changes in GHSR1a activity, have significant impact on energy homeostasis and body weight.

2.4 SGAs increase GHSR1a signalling independent of circulating ghrelin levels – a causal role in SGA-induced obesity: Circulating ghrelin is increased after olanzapine, clozapine and risperidone treatment in some individuals with schizophrenia [48, 49]; a result echoed in pre-clinical rodent models [12, 50]. In rats, olanzapine increases plasma ghrelin levels across a range of dosages [51, 52]. However, the fact that SGA-induced obesity is not always associated with hyperghrelinemia in humans and rodent models cannot be ignored. Indeed, several studies report

restored ghrelin homeostasis after 2-weeks of olanzapine and clozapine treatment, even though weight gain associated with hyperphagia can continue during the first 12 to 16 months of treatment [53-55]. This result is also observed in the pre-clinical rat model, as plasma ghrelin is increased after 8 days of olanzapine treatment, but declines to normal levels after 16 days, despite the continued progression of hyperphagia and weight gain in these rats [12, 50]. Therefore, drug effects on ghrelin secretion, both directly from its origin in the stomach or indirectly via vagal efferent commands from the brain [14], cannot be the only mechanism by which SGAs increase hypothalamic ghrelin signalling and stimulate obesity. We recently reported that olanzapine increases hypothalamic GHSR1a protein (18-28%) and mRNA (64-92%) expression, independent of treatment duration [12]. Olanzapine increases hypothalamic GHSR1a expression, and causes hyperphagia and body weight gain throughout the early (1 day), middle (14 days) and late (36 days) stages of treatment in rats, even though hyperghrelinaemia is only evident during the early treatment period [12]. Furthermore, olanzapine increases GHSR1a, phosphorylated AMPK and NPY, and decreases POMC expression in the hypothalamus [27, 51, 56]. The GHSR1a has an unusually high level of constitutive activity, ie: can be active in the absence of an agonist [57]. Therefore, it is possible that up-regulated GHSR1a by olanzapine increases obesogenic signalling pathways in the hypothalamus independent of circulating ghrelin levels.

In order to determine whether the increased GHSR1a levels are secondary to the elevated food intake induced by olanzapine, GHSR1a levels were measured in a pair-feeding experiment, where food intake of the olanzapine-treated female rats was clamped at the same amount as control rats [12]. As expected, pair-fed rats did not exhibit body weight differences between olanzapine and control groups, but olanzapine still increased GHSR1a protein and mRNA expression, and up-regulated levels of NPY and AgRP, as well as their transcription factors FOXO1, pCREB and BSX, [12]. Therefore, increased GHSR1a and downstream signalling in pair-fed rats, a paradigm that removes excessive food intake and weight gain as confounding factors, demonstrates a causal role for GHSR1a upregulation in olanzapine-induced weight gain rather than a consequence of increased

food intake or obesity [12]. In addition, direct delivery of a GHSR1a antagonist (D-Lys3-GHRP-6) to the brain inhibited GHSR1a signalling and prevented olanzapine-induced hyperphagia in rats [12]. Overall, increased GHSR1a signalling may cause the initial disruption to the primary hypothalamic appetite signalling pathways (GHSR1a/pAMPK/NPY/AgRP/POMC, Figure 1) that leads to hyperphagia and obesity during long-term treatment.

3. The 5-HT2cR and interaction of 5-HT2cR with GHSR1a in SGA-induced obesity

A number of studies have examined the involvement of 5-HT2cR in feeding behaviour. Agonists of 5-HT2cR, lorcaserin and mCPP, decrease food intake [58-60], while antagonists of 5-HT2cR, RS102221 and TMFPP (a 5-HT2cR and 5-HT1B receptor antagonist) significantly increase food intake [61]. In addition, obesity and over-eating behaviour has been observed in 5-HT2cR knock-out mice [62].

3.1 The 5-HT2cR is associated with SGA-induced obesity: pharmacogenetic and proteomic evidence. Evidence over the past decade has consistently identified an involvement of the 5-HT2cR in SGA-induced obesity [reviewed in 63]. Pharmacogenetic studies have revealed that a 5-HT2cR promoter polymorphism (-759 C/T) is associated with weight gain induced by antipsychotics. For example, in a sample of Chinese drug-naive schizophrenia patients 22% of subjects carrying the -759T allele had substantially lower weight gain than patients without the allele, following 10 weeks of treatment with SGAs [64]. Similarly, in a Caucasian group of first episode schizophrenia patients, the body weight gain in people with the genetic -759 C/T variant allele was also significantly lower after long-term (9 months) antipsychotic treatment [65]. Some negative reports have also emerged, in which body weight change after antipsychotic treatment was not related to 5-HT2cR-759C/T polymorphisms [66, 67]. This conflict in findings may be due to previous polypharmacy or differences in the ethnic origin of the participants, as subjects in one study included treatment-resistant individuals who had previously received high doses of traditional

antipsychotics [67], while the other report did not delineate ethnic origin within its sample population [66].

Olanzapine and clozapine have potent 5-HT2cR antagonist properties (Kd = 4.8 and 4.1nM) compared to other SGAs, based on the final concentration of radioligand [68] and the liability of clozapine and olanzapine-induced weight gain is higher than quetiapine (5-HT2cR Kd = 3500 nM), risperidone (5-HT2cR Kd = 32 nM) and aripiprazole (a 5-HT2cR partial agonist) [68, 69]. Another study reported that olanzapine and clozapine have higher 5-HT2cR binding affinities than haloperidol (Ki = 7.8, 10, >5000 nM for olanzapine, clozapine and haloperidol, respectively) [70]. 5-HT2cR antagonism is correlated with an increased risk of weight gain (rs=45%, $p<0.05$) and the morbidity rate associated with Type 2 diabetes mellitus (rs=90%, $p<0.05$) [71]; a result echoed in another study reporting that 5-HT2cR affinities for 17 typical and SGA drugs were significantly correlated with weight gain (rs=-0.49; $p<0.05$) [72]. We have previously shown that olanzapine significantly decreases 5-HT2cR binding density in the rat brain, demonstrating a strong effect of this drug on central 5-HT2cRs [73]. The literature linking 5-HT2C to SGA obesity is vast, but the causal relationship between 5-HT2cR antagonism and the weight gain liability of SGAs requires further investigation. We posit that SGA antagonism of the 5-HT2cR disrupts the normal inhibitory action of this receptor on the GHSR1a, resulting in upregulated orexigenic GHSR1a signalling pathways (discussed in the section below).

3.2 5-HT2cR interacts with GHSR1a to reduce GHSR1a signalling and food intake: Interactions between the serotonin and ghrelin signalling pathways in the brain have been reported in previous research. For example, GHSR1a activity is inhibited by serotonin, *in vivo* [17], and administration of a 5-HT2cR agonist inhibits ghrelin-induced food intake [17, 74]. Decreased 5-HT2cR mRNA expression is observed in GHSR1a knock-out mice, while the acute central administration of ghrelin increased 5-HT2cR mRNA expression in the amygdala and dorsal raphe

253 [75]. Moreover, the intra-hypothalamic administration of serotonin and a 5-HT2cR agonist (2,5-
254 dimethoxy-4-iodoamphetamine) effectively blocked ghrelin's orexigenic effects in rats [17]. NPY
255 (the GHSR1a downstream orexigenic signal) is also regulated by the 5-HT2cR, as administration of
256 the selective 5-HT2cR agonist, WAY-629, suppressed NPY mRNA expression in mice [76].
257 Another 5-HT2c/1B receptor agonist, mCPP, decreases NPY secretion in the hypothalamic PVN
258 and subsequently induced suppression of food intake [77]. Therefore, there is an interaction
259 between orexigenic GHSR1a, ghrelin, NPY and serotonin 5-HT2cR signalling in the brain, and a
260 functional effect of 5-HT2cR blockade on appetite through ghrelin signalling. Interestingly, the 5-
261 HT2cR can regulate GHSR1a signalling. The 5-HT2cR interacts with the GHSR1a to form a
262 heterodimer that inhibits ghrelin signalling [74]. Schellekens and colleagues used flow cytometry
263 fluorescence resonance energy transfer (fcFRET) to demonstrate the heterodimer between the
264 GHSR1a and 5-HT2cR in human embryonic kidney (HEK293A) cells [16, 74]. 5-HT2cR and
265 GHSR1a are also colocalized in rat hypothalamic and hippocampal neurons [74]. Furthermore, we
266 have found that olanzapine reduces 5-HT2cR and GHSR1a dimerization in a dose-dependent
267 manner in hypothalamic NPY neurons [78]. The GHSR1a is mainly localized in the plasma
268 membrane under resting conditions [79]. When exposed to ghrelin, an increase in 5-HT2cR and
269 GHSR1a dimer co-internalization can occur [16], preventing GHSR1a activity at the cell surface.
270 Functionally, 5-HT2cR and GHSR1a dimerization decreases GHSR1a-induced intracellular Ca^{2+}
271 signalling, while 5-HT2cR antagonism increases Ca^{2+} signalling [16]. Overall, the GHSR1a
272 interaction with 5-HT2cR appears to reduce ghrelin signalling that would lead to reduced food
273 intake. Therefore, 5-HT2cR antagonists (such as obesogenic SGAs) may block the inhibitory effect
274 of the 5-HT2cR on the GHSR1a to increase orexigenic signalling. Considering the co-localisation
275 of 5-HT2cR and GHSR1a receptors in multiple hypothalamic nuclei, alterations of the normal
276 interaction between these receptors may have wide-spread effects in the brain to alter energy
277 balance [74, 80-82].
278

Although the existence of a 5-HT2cR and GHSR1a heterodimer has been demonstrated, the exact molecular interaction between these receptor protein structures is unknown and requires further investigation. GHSR1a is a GPCR that contains in 15 structural fragments: 7 α-helix hydrophobic transmembrane (TM I-VII) domains, 6 loops (three intra- and extracellular), and 2 terminal segments. TM II and III are considered the ligand activation domains. Both endogenous and non-endogenous ligand binding causes a conformational change in the GHSR1a molecular structure, characterized by a reciprocal rearrangement of the α-helices, with vertical seesaw movements of TM VI and TM VII around their central proline residues. This alteration can cause the intracellular ends of TM VI and TM VII to move away from the center of the receptor toward TM III, exposing the sites subsequently recognized by G-proteins and β-arrestin [83]. The constitutive activity of GHSR1a is affected by an aromatic cluster formed by three amino acid residues (Phe VI:16, Phe VII:06, and Phe VII:09) on the inner face of the extracellular ends of GHSR1a TM helices VI and VII, as reported in a cell-based mutagenesis study [83, 84]. It is the formation of the hydrophobic core between TM helices VI and VII that ensures proper docking of the extracellular end of TM helices VII into VI, mimicking agonist activation and stabilizing the receptor in active conformation. Specific residues in the vicinity of this cluster orchestrate microswitches that are critical for GHSR1a activation levels in the absence of a ligand (constitutive activity). The 5-HT2cR is also a GPCR with 7 TM helices. Alterations to the amino acid sequence in the editing site located in the second intracellular loop of the 5-HT2cR produce isoforms of this receptor (Figure 1) [85-87]. Interestingly, the GHSR1a dimerizes with the unedited 5-HT2cR(INI) isoform, but not with the partially edited 5-HT2cR (VSV) isoform [16]. Therefore, the 5-HT2cR second intracellular loop may interact with the GHSR1a transmembrane helices VI and VII to form a heterodimer that may inhibit the constitutive activity of GHSR1a and subsequent orexigenic signalling. Given that olanzapine consistently upregulates GHSR1a and is a 5-HT2cR antagonist, blockade of 5-HT2cR may decrease the normal inhibition of this receptor on the constitutive activity of GHSR1a. It is conceivable that novel pharmacological agents that target these transmembrane helices, or that

increase the affinity of the 5-HT2cR to the GSHR1a, may be useful therapies to prevent or attenuate SGA-induced weight gain side-effects.

3.3 Use of a 5-HT2cR agonist to prevent / attenuate SGA-induced obesity

The recent U.S. Food and Drug Administration (FDA) approval of the 5-HT2cR receptor agonist lorcaserin for the treatment of obesity represents a new therapeutic drug class available to the clinic. A randomized, double-blind, placebo-controlled clinical trial over of 2,200 over-weight and obese subjects revealed 5-10% weight loss with lorcaserin sustained over 1-year [88]. Lorcaserin (1 – 2mg/kg SC b.i.d.) treatment for 28 days significantly reduced the percentage of body weight gain compared to vehicle-treated controls in a diet-induced obese rat model, attributed largely to a reduction in body fat mass [89]. Lorcaserin is also effective at attenuating ghrelin-induced food intake in mice [74], demonstrating a potential interaction between lorcaserin and the ghrelin signalling system. Furthermore, a case study reported weight loss with lorcaserin in a schizophrenia patient treated with olanzapine [90]; however, the mechanisms are unknown and further studies are required. The potent 5-HT2cR antagonist property of olanzapine could be responsible for disrupting the normal inhibitory tone of the 5-HT2cR on the GHSR1aby reducing inhibitory 5-HT2cR/GHSR1a interactions (Figure 2). Interestingly, another 5-HT2cR agonist, vabicaserin, recently demonstrated antipsychotic efficacy in a Phase II trial of schizophrenia patients, with no weight gain and minimal extrapyramidal side-effects [91]. Therefore, co-treatment of olanzapine with a 5-HT2cR agonist (lorcaserin or vabicaserin) is a promising novel possibility for restoring 5-HT2cR activity and preventing the initial disruption to GHSR1a-induced appetite signalling caused by olanzapine. In addition, POMC neurons also regulate food intake and express 5-HT2cR. Only 8% POMC neurons express GHSR1a suggesting that 5-HT2cR can regulate POMC independent of GHSR1a. The anti-obesity effect of 5-HT2cR agonist may involve both hypothalamic NPY and POMC neurons, but via different mechanism. In addition, there is no literature reporting the effect

of obesogenic antipsychotic drugs on genetic mutant mouse models on 5-HT2cR and GHSR1a, which may help to verify the specificities of these receptors in SGA-induced obesity.

4. Concluding Remarks and Future Perspectives

Both antagonism of serotonin 5-HT2cR and activation of GHSR1a signalling have been identified as the main causes of SGA-induced obesity. Activation of the GHSR1a plays an important role in SGA-induced obesity through intracellular signalling pathways (AMPK-CPT1-UCP2) that increase orexigenic NPY and AgRP, and suppress anorexigenic POMC signalling in the hypothalamus [23, 25, 26]. The 5-HT2cR plays a pivotal regulatory role in ghrelin-mediated appetite signalling. The 5-HT2cR dimerizes with the GHSR1a to inhibit its orexigenic activity, while 5-HT2cR antagonism reduces the dimerization and increases GHSR1a-induced food intake [16, 74]. Obesogenic SGAs, including olanzapine, clozapine, and risperidone, possesses potent 5-HT2cR antagonist properties [92]. Therefore, 5-HT2cR antagonism by SGAs may disinhibit the GHSR1a to increase orexigenic signalling. Unfortunately, the molecular mechanisms linking 5-HT2cR antagonism and GHSR1a in SGA-induced obesity remain unclear. The constitutive activity of GHSR1a is highly influenced by an aromatic cluster on the inner face of the extracellular ends of TM helices VI and VII [83]. The residues of the 5-HT2cR in the editing cassette located in the second intracellular loop of 5-HT2cR [85] may interact with this cluster to inactivate its conformation. Further investigation into this mechanism is warranted. In addition, combined treatment of a 5-HT2cR agonist with SGAs may restore inhibitory control of GHSR1a by the 5-HT2cR. Lorcaserin, a FDA approved anti-obesity 5-HT2cR receptor agonist drug, attenuates ghrelin-induced food intake [74]. Therefore, co-treatment of SGAs with lorcaserin may prevent the disruption to GHSR1a-induced appetite signalling caused by SGAs. In summary, antagonism of the 5-HT2cR by SGAs may reduce GHSR1a interaction with the 5-HT2cR and activate ghrelin signalling to stimulate feeding behavior. The 5-HT2cR and its interaction with GHSR1a could be clinically relevant for the treatment of SGA-induced obesity and

a valuable target for the design of new compounds that remove 5-HT2cR antagonist properties of SGAs to prevent obesity.

Acknowledgments

This work was supported by the National Health and Medical Research Council of Australia (APP1027493 to X.F.H.) and Fellowship (NHMRC 573441 to Y.H.Y.).

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Figure legends:

Figure 1. Schematic drawing of ghrelin signalling pathway and interaction with 5-HT2cR in the hypothalamus. Ghrelin binds to the ghrelin receptor (GHSR1a), triggering phosphorylation of 5' AMP-activated protein kinase (AMPK) that activates carnitine palmitoyl transferase 1 (CPT1), subsequent fatty acid shuttling into the mitochondria and uncoupling protein-2 (UCP2) activity. Transcriptional factors, forkhead box O1 (FOXO1) and phospho-cAMP-response element binding protein (pCREB) translocate to the nucleus and bind to their response element on the DNA. Brain-specific homeobox (BSX) interacts with FOXO1 and pCREB to initiate expression of neuropeptide Y (NPY) and agouti-related peptide (AgRP). The serotonin 5-HT2c receptor (5-HT2cR) is a G protein-coupled receptor with seven transmembrane domains. The 2nd intracellular loop contains an editing site. At this site, three amino residues (shown as red dots) can exist in an unedited state (5-HT2cR-INI isoform) or a partially edited state (5-HT2cR-VSV isoform) that either enables or prevents heterodimer formation (for example with the GHSR1a), respectively. The unedited 5-HT2cR-INI isoform can form a heterodimer with the GHSR1a transmembrane helices VI and VII that inhibits this orexigenic signalling pathway.

Figure 2. The primary mechanism of antipsychotics-induced obesity. This figure depicts how second generation antipsychotics (SGAs) affect the hypothalamic circuit in regulating body weight. The ghrelin receptor (GHSR1a) is expressed on NPY/AgRP neurons of the Arc, the PVN and VMH. GHSR1a orexigenic signalling is inhibited by serotonin 2c receptors (5-HT2cR) through heterodimerisation (described in Figure 1). SGAs are 5-HT2cR antagonists and blockade of this receptor may release inhibition of hypothalamic GHSR1a signalling. Given the complexity of hypothalamic appetite signalling, alterations in signalling in this region would have wide-spread effects on multiple systems and physiological outcomes leading to body weight gain and obesity side-effects. Abbreviations: neuropeptide Y (NPY), agouti-related peptide (AgRP), pro-opiomelanocortin (POMC),gamma-Aminobutyric acid (GABA), melanocortin 3 and 4 (MC3 and MC4), α melanocortin-stimulating hormone (α MSH), tryrotropin- and corticotropin-releasing

622 hormones (TSH and CRH), steroidogenic factor 1 (SF1), brain-derived neurotrophic factor (BDNF),
623 melanin-concentrating hormone (MCH). paraventricular nucleus (PVN), ventromedial
624 hypothalamus (VMH), dorsomedial hypothalamic nucleus (DMH), lateral hypothalamus (LH), 3rd
625 ventricle (3V), arcuate nucleus (Arc), nucleus tractus solitarius (NTS), parabrachial nucleus (PBN),
626 sympathetic nervous system (SNS), brown adipose tissue (BAT), white adipose tissue (WAT).
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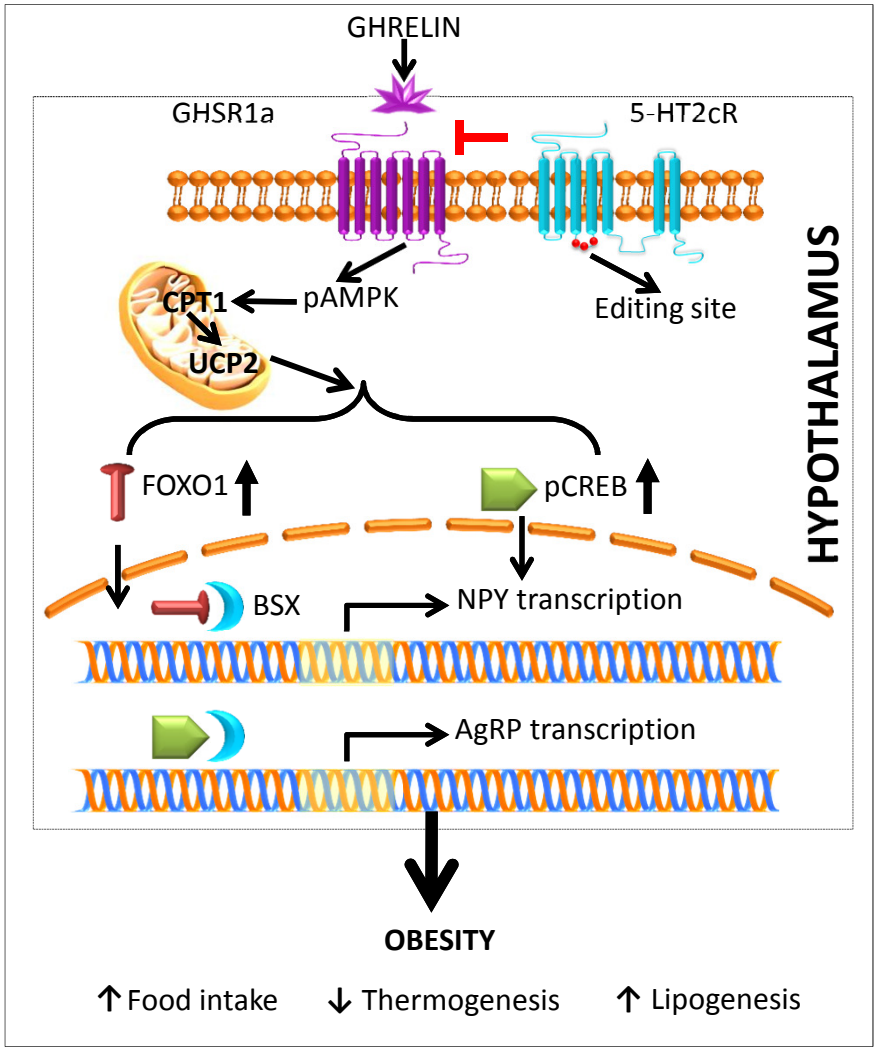
Figure 1


GHRELIN
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AgRP transcription
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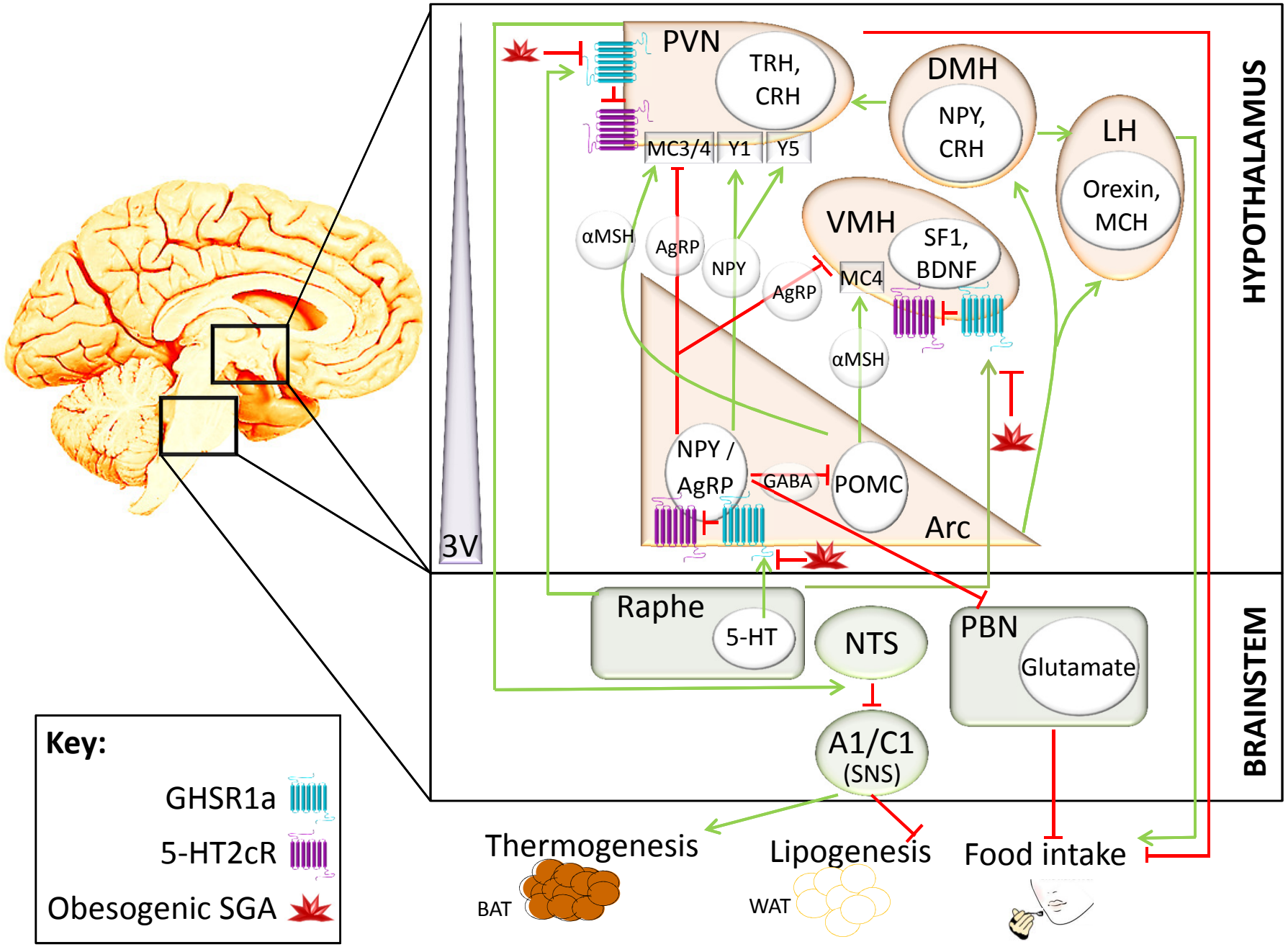
↓ Thermogenesis
↑ Lipogenesis


HYPOTHALAMUS
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SF1, BDNF
MC4
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AgRP
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GABA
POMC
Arc
3V
Raphe
5-HT
NTS
PBN
Glutamate
A1/C1 (SNS)
Thermogenesis
BAT
Lipogenesis
WAT
Food intake
Key:
GHSR1a
5-HT2cR
Obesogenic SGA