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Review Article

The role of Y_1 receptors in regulating food intake

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Introduction

The hypothalamus is a pivotal center for energy homeostasis. Within the past few decades, investigations have emerged from various laboratories that implicate many critical factors in the process of feeding regulation. Neuropeptide Y (NPY), the most potent orexigenic peptide identified thus far, is one of these factors (1-3). Chronic administration of NPY into the brain results in body weight gain with hyperphagia, reduces energy expenditure and increases lipogenic activity in the liver and adipose tissue (4, 5). In addition, NPY-deficient *ob/ob* mice are less obese and have reduced food intake when compared with *ob/ob* mice (6). From these data, it has been inferred that NPY is a major regulator of energy balance.

The presence of at least 6 distinct subtypes of NPY receptors has been described, and 5 of them (Y_1, Y_2, Y_4, Y_5 and Y_6) have been cloned (7-21). Accumulating evidence shows that NPY-mediated feeding might be regulated by multiple hypothalamic NPY receptor subtypes (21-25). Of these receptors, Y_1 and Y_5 are of particular interest in feeding regulation. Based on the correlation between the *in vitro* functional or binding activities of different peptide agonists and their potencies in stimulating food intake in rodent models, Y_5 receptors are believed to be involved in feeding regulation (17). With respect to Y_1 receptors, Y_1-specific antagonists have been shown to suppress feeding behavior, indicating that Y_1 receptors are also involved in feeding regulation (26-36).

Orexigenic effects of NPY and related peptide analogues

NPY is a 36-amino acid polypeptide belonging to the pancreatic polypeptide family. This family consists of NPY, peptide YY (PYY) and pancreatic polypeptide (PP) (1). Using various NPY analogues, including PYY and PP, 2 major types of NPY receptors, namely Y_1 and Y_2 receptors, were defined (37, 38). Both N and C terminals of NPY are important for the activation of Y_1 receptors (39). In contrast, Y_2 receptors recognize the C-terminal portion of NPY (39). Because the intracerebroventricular (i.c.v.) injection of the Y_2-selective agonist NPY(13-36) was shown not to stimulate food intake, the involvement of Y_1 receptors in NPY-induced feeding behavior was considered (40, 41). A recent study employing highly selective Y_1 agonists strongly supports this possibility (42). NPY2-36 and PYY3-36 are less potent than intact NPY and PYY for most functions of Y_1 receptors (8, 17, 25); however, they are more effective than NPY in stimulating feeding behavior (17, 25, 40, 41). These data suggest that Y_1-like receptors that are distinguishable from Y_1 receptors may exist in the hypothalamus to regulate food intake. In order to find Y_1-like receptors, other subtypes of NPY receptors such as Y_4, Y_5 and Y_6 have been identified (14-21). Although human Y_4 receptors have shown a relatively high homology to human Y_1 receptors (42%), Y_4 is a receptor for PP and not for NPY (14-16). Therefore, Y_4 receptors are not considered Y_1-like in NPY-mediated feeding regulation. Y_6 receptors are most structurally related to Y_1 receptors. Murine Y_6 receptors share 60% of the amino acid identity of murine Y_1 receptors, and the affinities of NPY ligands for murine Y_6 receptors have been reported as Y_1-like (19). Therefore, the Y_6 receptor might be a type of Y_1-like feeding receptor in mice. However, in rats and mice, PYY3-36 is the most potent feeding stimulant despite the fact that rats (and primates) lack functional Y_6 receptors (20, 21); this indicates that Y_6 receptors are not obligatory feeding receptors. Y_5 receptors are most likely to be Y_1-like feeding receptors, as evidenced by the correlation between the *in vitro* functional or binding activities of different peptide agonists and their potencies for stimulation of food intake in rodent models (17). However, PYY3-36 has been shown to be

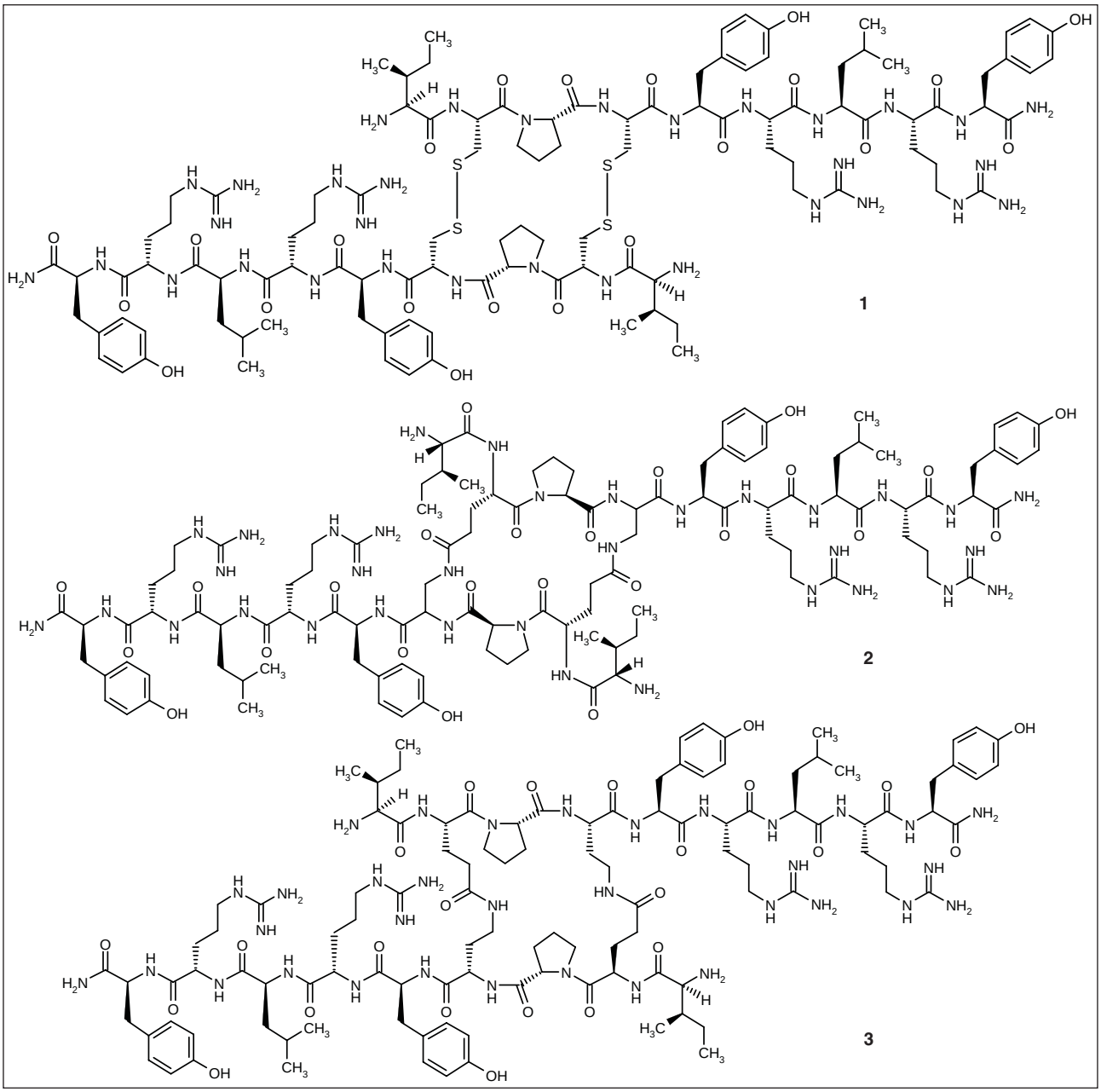

Fig. 1. Peptide Y_1 antagonists with a dimeric structure.

less potent than NPY at the Y_5 receptor in *in vitro* studies and still significantly stimulates food intake in Y_5 receptor-deficient mice (17, 22, 25). Although the presence of the Y_1-like receptor is still controversial, these findings show that NPY-induced feeding is regulated by multiple NPY receptors. Of the receptors, Y_1 may be one of the key players in NPY-mediated feeding regulation.

Anorectic effects of peptide Y_1 antagonists

The first potent Y_1 antagonists, **1** (383U91) and **2** (1229U91/GW-1229), are a series of peptide analogues with a dimeric structure (Fig. 1) (26). Compound **2** has shown a K_i value of 0.041 nM to human Y_1 receptors and has demonstrated potent Y_4 agonistic activity (K_i = 0.33 nM) (29). However, another Y_4 selective agonist, rat PP, failed to stimulate feeding behavior, suggesting that typical Y_4 receptors are not involved in feeding regulation. Thus, **2** is a useful tool for evaluating the role of Y_1 receptors in feeding regulation in terms of solubility and toxicity. Injection of **2** (30 mcg) i.c.v. in male Sprague-Dawley (SD) rats completely inhibited NPY (5 mcg)-induced food intake and physiological feeding behavior after overnight fasting in a dose-dependent manner (25). In addition, the

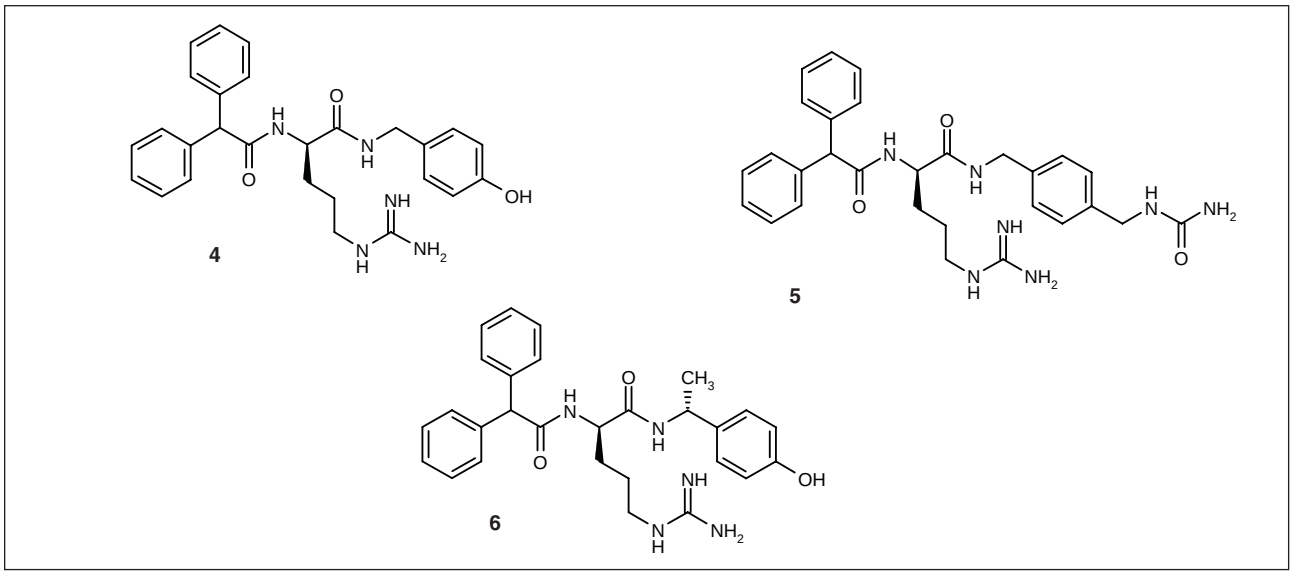

Fig. 2. BIBP-3226 and analogues.

peptide Y_1 antagonist, **3** (NNC-58-0036, pK_i = 10.12 for human Y_1, pK_i = 9.11 for human Y_4), inhibited NPY- and fasting-induced feeding in rhesus monkeys (Fig. 1) (43). These results indicate that Y_1 receptors play a key role in NPY-induced food intake and that physiological feeding behavior after overnight fasting may be largely regulated by NPY via Y_1 receptors in rodents and primates.

Anorectic effects of nonpeptide Y_1 antagonists

The anorectic effects of several nonpeptide Y_1 antagonists were confirmed after i.c.v. or systemic administration. The first class of nonpeptide Y_1 antagonists includes Karl-Thomae's Y_1 antagonist, **4** (BIBP-3226), and its analogue, **5** (BIBO-3304) (Fig. 2) (30, 31, 44). Although the anorectic effects of BIBP-3226 are controversial (45), a more potent analogue, **5** (IC_{50} = 0.69 nM for human Y_1), was shown to significantly inhibit NPY-induced food intake after i.c.v. dosing with 30 mcg (31). Compared with **4**, the Y_1-inactive (*S*)-enantiomer of **5** (structure not shown) had no effects in the same feeding paradigm.

A series of arylindole and benzimidazole derivatives from Eli Lily includes very potent Y_1 antagonists (28, 46). One of the representative antagonists, **7** (LY-357897, K_i = 0.75 nM for human Y_1), was shown to inhibit NPY-induced food intake after i.c.v. administration in a dose-dependent manner (11-26 nmol/head) (Fig. 3) (28).

Aminopyridine derivatives are the first class of Y_1 antagonists with oral bioavailability and brain permeability. After oral administration, **8** (K_i = 0.26 nM for human Y_1) and **9** (K_i = 1.4 nM for human Y_1) inhibited NPY-induced food intake as well as spontaneous feeding in rodents (Fig. 3) (32, 36, 47). The *in vivo* specificity of **9** was also validated in Y_1-deficient mice (36). Other structural classes of compounds with brain permeability were recently reported. Bristol-Myers Squibb's dihydropyridine derivative, **10** (BMS-193885, K_i = 3.3 nM for human Y_1), and Pfizer/Neurogen's purine derivative, **11** (CP-671906, K_i = 4 nM for human Y_1), were shown to suppress several types of feeding behavior after systemic administration (Fig. 3) (33-35).

The structural diversity of several potent Y_1 antagonists validates the role of Y_1 receptors in NPY-mediated feeding behavior.

Genetic inactivation of Y_1 receptors

NPY-receptor-deficient mice are also informative tools for interpreting the role of Y_1 receptors. NPY- and fasting-induced food intake were shown to be significantly suppressed in Y_1-receptor-deficient (Y_1–/–) mice (23, 25). These observations represent additional important evidence demonstrating the pivotal role of Y_1 receptors in feeding regulation. Moreover, Y_1 receptors might modulate the actions of other NPY receptors including Y_5 receptors. Increases in feeding induced by various peptides from the NPY family, which have weak binding affinity for Y_1 receptors, were reduced in Y_1–/– mice when compared with that in wild-type mice (25). A potential synergistic involvement of both Y_1 and Y_5 receptors in mediating feeding behavior is consistent with a recent report showing that neurons positive for Y_5 receptors also express Y_1 receptors in the hypothalamus (49). Y_1 receptors might modulate other NPY receptor functions as well as direct the stimulation of NPY-induced feeding.

Interestingly, Y_5 receptor-deficient mice (Y_5–/–) with functioning Y_1 receptors have late-onset obesity with hyperphagia (22). Although details of the occurrence of hyperphagia still need to be addressed, the overcompensation of Y_1 receptors in Y_5–/– mice may be one of the

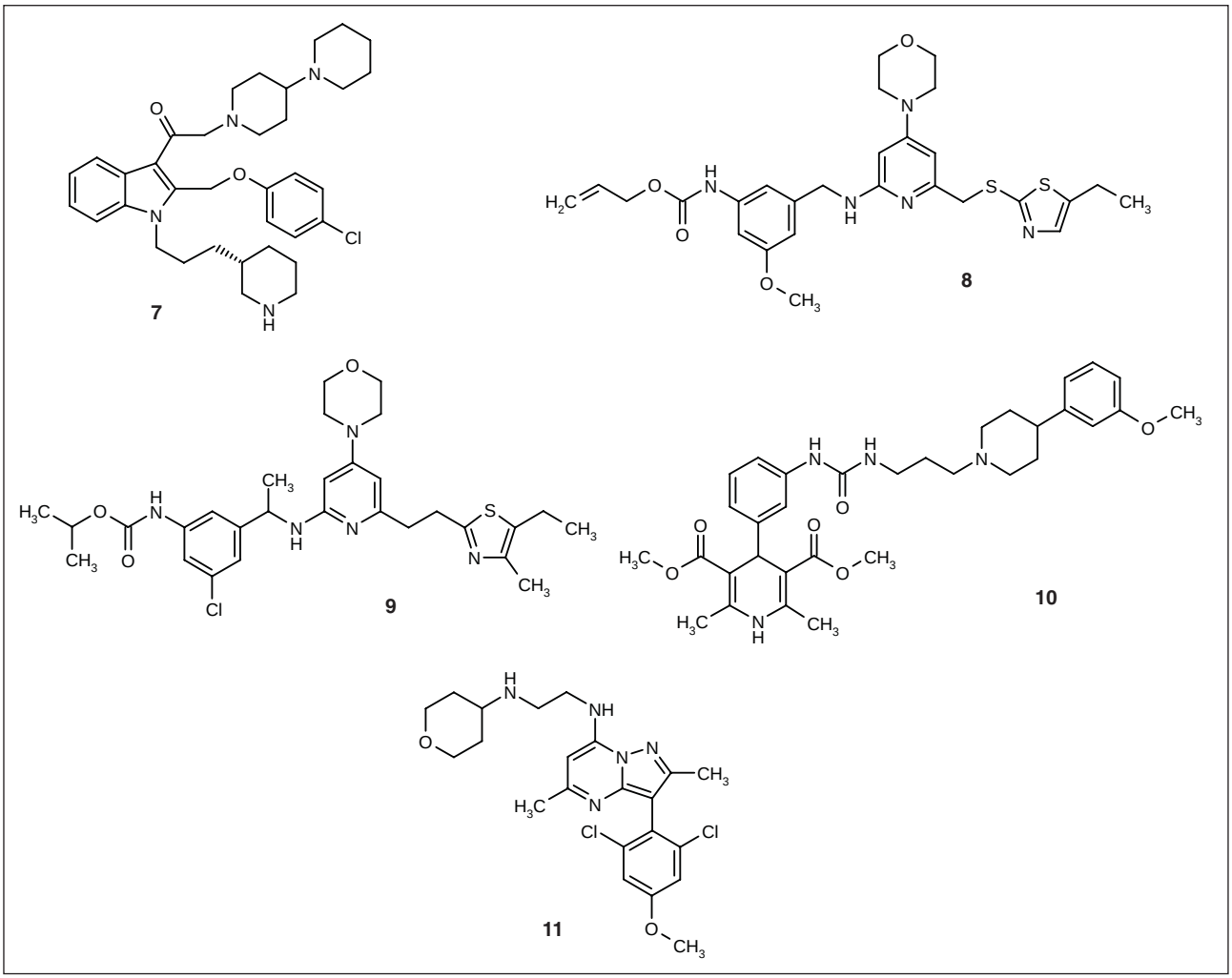

Fig. 3. Other Y_1 antagonists.

possible mechanisms to explain hyperphagia in the late-onset obesity.

Interactions with other feeding regulators

Leptin is secreted by adipocytes and regulates energy homeostasis. The lack of leptin signals causes obesity in rodents and humans (50-52). Increased hypothalamic expression of NPY and its mRNA has been reported in leptin signaling-deficient rodents such as Zucker fatty rats, *db/db* and *ob/ob* mice and is considered to be an important cause of obesity (53, 54). In addition, NPY-deficient *ob/ob* mice are less obese and have reduced food intake when compared with *ob/ob* mice (6). Thus, NPY is at least partly responsible for the abnormality of leptin signal-deficient obesity. It is noteworthy that food intake was suppressed to a greater extent after the i.c.v. administration of **2** in obese Zucker rats than in lean Zucker rats without abnormal behavior (55). Other nonpeptide Y_1 antagonists also suppressed spontaneous feeding in Zucker fatty rats and *db/db* mice (32, 36). In contrast, Y_5 antagonists have failed to inhibit spontaneous feeding behaviors in leptin signal-deficient rodents (47, 48). These findings shows that the hyperphagia in leptin signal-deficient models might be due to the abnormal overactivation of Y_1 receptors. In addition, these findings suggest the possibility of the participation of Y_1 receptors in pathophysiological feeding in humans because the leptin deficiency may cause early-onset obesity with hyperphagia (52).

The melanocortinergic system is an interesting pathway involved in hyperphagia. Genetic mutations of POMC (ligand), MC4-R (receptor) and PC-1 (processing enzyme) were also reported to elicit hyperphagia and obesity in humans (56-59). Injection of MC4-R antagonists i.c.v. was shown to stimulate food intake in rats, and Y_1 antagonists were shown to inhibit MC4-R antagonist-induced food intake (60, 61). Although such mutations in the melanocortinergic system are rare in humans with

obesity and hyperphagia, such as in the case of leptin deficiency, they provide additional evidence that Y_1 receptors may be involved in pathophysiological feeding in humans as well as in rodents.

Interestingly, Y_1 antagonists were also shown to suppress ghrelin-, orexin- and MCH-induced food intake (62-66). Y_1 receptors might be also involved in feeding behaviors evoked by other neuropeptides; however, the precise roles of these orexigenic neuropeptides in physiological feeding are still unclear, especially in humans.

In contrast, Y_1 antagonists do not show any significant effects on galanin- and noradrenaline-induced food intake (29, 31). Because several behavioral changes affect feeding regulation, it might be difficult to completely exclude the influence of behavioral changes such as mood, arousal, taste aversion, and so on. However, the selectivity of Y_1 antagonists in several feeding paradigms may support the direct participation of Y_1 receptors in feeding regulation.

Taken together, Y_1 receptors play a central role in several feeding pathways as well as in the modulation of NPY receptor subtypes.

Clinical studies

Of the Y_1 antagonists reported thus far, Pfizer/Neurogen's, NGD-95-1 (structure unknown) and Astra's, H-409/22 (**6**), have reached the clinical trial stage (Fig. 2). A phase Ib trial of NGD95-1 was discontinued because patients taking the drug developed elevated liver enzyme levels (67). H-409/22 has no oral bioavailability and brain permeability and, therefore, has only been administered as an i.v. infusion for the treatment of angina (68). Although H-409/22 blocked the elevation of blood pressure produced by a bolus dose of NPY, the clinical trial of H-409/22 was suspended due to a lack of clinical effect. However, plasma NPY levels were significantly increased during the i.v. infusion of the compound, suggesting that peripheral Y_1 receptors may also function as clearance receptors (68).

Future perspectives

The key role of Y_1 receptors in feeding regulation is now much clearer based on the observations made in many studies. Thus, the potential of Y_1 antagonists as feeding suppressants for clinical treatment seems promising. However, it was also reported that the anorectic effects of potent feeding suppressants were ameliorated within a few days, possibly due to a compensation by several factors that regulate feeding behavior (69, 70). A reduction in feeding suppression by **8** was also observed in Zucker fatty rats (71). Interestingly, during chronic dosing of **8,** body weight gain was lower in the treatment group than in the vehicle-treated group, even when food intake returned to control levels. These results suggest the presence of additional effects of Y_1 antagonists in energy homeostasis. It is well known that i.c.v. NPY infusions cause obesity, accompanied by hyperphagia, as well as a reduction in energy expenditure; however, the involvement of NPY receptor subtypes is unclear (4, 5). Therefore, other functions of Y_1 receptors in energy homeostasis need to be elucidated in order to correctly interpret the potential of Y_1 antagonists as therapeutic agents.

Conclusions

Accumulating evidence shows that Y_1 receptors have pivotal roles in feeding regulation. However, recent research is providing us with knowledge of many factors that are involved in feeding. The role of Y_1 receptors in the complicated pathways is still an uncharted area, especially in humans. Knowledge of the orexigenic map could be of importance for correctly addressing the role of Y_1 receptors in regulating food intake.

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