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Neuropeptide Y receptors as targets for anti-obesity drug development: perspective and current status

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Abstract

Neuropeptide Y is a widely distributed neuropeptide that elicits a plethora of physiological effects via interaction with six different receptors (Y_1–y_6). Recent attention has focused on the role of neuropeptide Y in the regulation of energy homeostasis. Neuropeptide Y stimulates food intake, inhibits energy expenditure, increases body weight and increases anabolic hormone levels by activating the neuropeptide Y Y_1 and Y_5 receptors in the hypothalamus. Based on these findings, several neuropeptide Y Y_1 and Y_5 receptor antagonists have been developed recently as potential anti-obesity agents. In addition, mice lacking neuropeptide Y, the neuropeptide Y Y_1 receptor or the neuropeptide Y Y_5 receptor have been generated. The data obtained to date with these newly developed tools suggests that neuropeptide Y receptor antagonists, particularly neuropeptide Y Y_1 receptor antagonists, may be useful anti-obesity agents. However, the redundancy of the neurochemical systems regulating energy homeostasis may limit the effect of ablating a single pathway. In addition, patients in whom the starvation response is activated, such as formerly obese patients who have lost weight or patients with complete or partial leptin deficiency, may be the best candidates for treatment with a neuropeptide Y receptor antagonist.

Keywords: Neuropeptide Y; Obesity; Neuropeptide receptor, neuropeptide Y; Anti-obesity agent

1. Introduction

Neuropeptide Y is a 36-amino acid neuropeptide that was discovered in 1982 in a directed search for C-terminally amidated peptides (Tatemoto et al., 1982). Neuropeptide Y is a member of the pancreatic polypeptide family; other members of this family are the structurally related peptides, peptide YY and pancreatic polypeptide (Tatemoto et al., 1982). Neuropeptide Y is widely distributed in both the central and peripheral nervous systems and is one of the most abundant neuropeptides known. In the periphery, neuropeptide Y is localized in post-ganglionic sympathetic neurons, adrenal medulla, enteric neurons, cardiac non-sympathetic neurons, certain non-adrenergic perivascular neurons and parasympathetic neurons (Grundemar and Hakanson, 1993; McDermott et al., 1993). In sympathetic neurons and adrenal medulla, the peptide is co-localized with the classical sympathetic neurotransmitter noradrenaline (Lundberg et al., 1982). In the brain, neuropeptide Y-containing neuronal cell bodies are found primarily in the locus coeruleus, the nucleus of the solitary tract and the arcuate nucleus of the hypothalamus. These neuropeptide Y-containing neuronal cell bodies also typically contain other neurotransmitters such as noradrenaline and send projections throughout the brain; hence, neuropeptide Y can be found in most brain regions, particularly in the cortex, hippocampus, thalamus, hypothalamus and brainstem (Chronwall et al., 1985).

Since it was one of the first neuropeptides to be discovered and is ubiquitously distributed, a great deal is now known about the biology of neuropeptide Y. Neuropeptide Y has been implicated in a wide variety of physiological effects; hence, neuropeptide Y and its receptors have sparked a great deal of basic research and drug development interest. This review will focus on the role played by neuropeptide Y and neuropeptide Y receptors in the regulation of energy homeostasis. The reader is referred to other excellent reviews for a more comprehensive discussion of the biology of neuropeptide Y (Cerda-Reverter and Larhammar, 2000; Grundemar and Bloom, 1997; Wettstein et al., 1995).

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2. Neuropeptide Y receptors

Neuropeptide Y, peptide YY and pancreatic polypeptide elicit their physiological effects by interacting with at least six distinct G protein-coupled receptors designated Y_1, Y_2, Y_3, Y_4, Y_5 and y_6 (Michel et al., 1998). With the exception of the neuropeptide Y Y_3 receptor, genes and/or cDNAs encoding each of these neuropeptide Y receptors have been cloned. In contrast to other families of G protein-coupled receptors, the neuropeptide Y receptors share only modest primary sequence homology (30–50%). In fact, some neuropeptide Y receptors are structurally more related to G protein-coupled receptors outside of the neuropeptide Y receptor family. The structural differences among neuropeptide Y receptors is beneficial to drug discovery efforts since compounds with high affinity for a particular neuropeptide Y receptor are less likely to interact with other neuropeptide Y receptors. In addition to having distinct amino acid sequences, each of the neuropeptide Y receptors is characterized by a unique pharmacological profile (Table 1) and a distinct tissue localization.

2.1. The neuropeptide Y Y_1 receptor

The neuropeptide Y Y_1 receptor was initially identified in early pharmacological studies as the neuropeptide Y receptor responsible for contraction of isolated vascular preparations (Grundemar and Bloom, 1997). In these studies, the neuropeptide Y Y_1 receptor was pharmacologically defined by its high affinity for neuropeptide Y and [Pro^{34}]-peptide YY and its low affinity for N-terminally truncated neuropeptide Y analogues (Table 1). Neuropeptide Y Y_1 receptor mRNA is abundantly expressed in many rat and human brain regions, including hypothalamic centers controlling energy homeostasis (Caberlotto et al., 1997; Parker and Herzog, 1999). Neuropeptide Y Y_1 receptor binding sites are also abundantly expressed in many rat brain regions, including the hypothalamus (Caberlotto et al., 1998b; Dumont et al., 1996). In contrast, levels of neuropeptide Y Y_1 receptor binding sites are very low throughout most of the human brain, perhaps due to instability of the receptor during the post-mortem interval (Caberlotto et al., 1997). In the periphery, neuropeptide Y Y_1 receptor mRNA is expressed primarily in rodent kidney, heart, spleen, skeletal muscle, lung, gastro-intestinal tract and vascular smooth muscle (Goumain et al., 1998; Nakamura et al., 1995), and in human kidney, heart, lung, colon, testis, adrenal gland, placenta, bone marrow and vascular smooth muscle (Wharton et al., 1993).

Table 1
Affinities of neuropeptide Y (NPY), peptide YY (PYY), pancreatic polypeptide (PP) and related peptides for the known neuropeptide Y receptors

Peptide	EC_{50} (nM)					
	rY_1	rY_2	rY_3	rY_4	rY_5	my_6
hNPY	0.14	1.2	1.8	>1000	0.96	1.9
PYY	0.70	0.58	7200	>1000	1.0	0.8
pNPY-(2–36)	3.4	1.6		>1000	1.2	1.4
pNPY-(3–36)	110	2.4		>1000	2.8	3.9
pNPY-(13–36)	300	2.2		>1000	20	16.1
[Pro^{34}] PYY	0.37	>1000		6.0	1.3	
hPP	150	>1000		0.037	1.4	>1000
rPP	>1000	>1000		0.060	170	>1000

Data are from Gerald et al. (1996), with the exception of the rat neuropeptide Y Y_3 receptor (rY_3) data which are from Higuchi et al. (1988) and the mouse neuropeptide Y y_6 receptor (my_6) data, which are from Mullins et al. (2000). Prefixes before peptide or receptor names are as follows: h=human, p=porcine, r=rat, m=mouse.

2.2. The neuropeptide Y Y_2 receptor

The neuropeptide Y Y_2 receptor was also identified in early pharmacological studies as the neuropeptide Y receptor responsible for regulation of noradrenaline release from sympathetic nerve terminals (Grundemar and Bloom, 1997). The neuropeptide Y Y_2 receptor is pharmacologically unique in that it has high affinity for N-terminally truncated neuropeptide Y analogues and low affinity for [Pro^{34}]peptide YY (Table 1). The neuropeptide Y Y_2 receptor is localized in several rat and human brain regions, including hypothalamic nuclei regulating energy homeostasis (Caberlotto et al., 1998a; Parker and Herzog, 1999). The neuropeptide Y Y_2 receptor is localized on neuropeptide Y-containing neurons in the brain, suggesting that this receptor is an autoreceptor (Caberlotto et al., 2000; King et al., 2000). In the periphery, the neuropeptide Y Y_2 receptor can be pharmacologically detected on the terminals of rat sympathetic, parasympathetic and sensory neurons, again functioning as an autoreceptor or heteroreceptor (Grundemar and Bloom, 1997). Negligible levels of neuropeptide Y Y_2 receptor mRNA are generally found in peripheral tissues (Gehlert et al., 1996), although low expression of neuropeptide Y Y_2 receptor mRNA has been reported in human and rat gastro-intestinal tract (Gehlert et al., 1996; Goumain et al., 1998).

2.3. The neuropeptide Y Y_3 receptor

The neuropeptide Y Y_3 receptor was originally characterized pharmacologically in bovine adrenal chromaffin cells and has subsequently been found in rat heart, brainstem, hippocampus, colon and lung (for references, see Michel et al., 1998). The distinguishing pharmacological feature of the Y_3 receptor is its much higher affinity for neuropeptide Y than for peptide YY (Table 1). Although pharmacological evidence supports the existence of the neuropeptide Y Y_3 receptor in rats and cows, there are no reports of this receptor in humans and the receptor has not yet been cloned. Cloning of this receptor will be required to unequivocally validate its existence and determine its physiological role.

2.4. The neuropeptide Y Y_4 receptor

The neuropeptide Y Y_4 receptor was initially identified by molecular cloning (Lundell et al., 1995). This receptor is pharmacologically characterized by its high affinity for pancreatic polypeptide and its low affinity for neuropeptide Y (Table 1; Lundell et al., 1995). Therefore, the neuropeptide Y Y_4 receptor is probably a pancreatic polypeptide receptor rather than a neuropeptide Y receptor. Neuropeptide Y Y_4 receptor mRNA is sparsely expressed in brain; expression is seen primarily in the brainstem, but also at low levels in other brain regions such as the hypothalamus (Parker and Herzog, 1999). In the periphery, the neuropeptide Y Y_4 receptor is expressed primarily in rat testis and lung (Lundell et al., 1996). A different pattern of peripheral expression is seen in humans, with expression primarily detected in colon, small intestine, prostate and pancreas (Lundell et al., 1996).

2.5. The neuropeptide Y Y_5 receptor

The neuropeptide Y Y_5 receptor was also initially identified by molecular cloning (Gerald et al., 1996). The neuropeptide Y Y_5 receptor is pharmacologically distinguished by its high affinity for both N-terminally truncated analogues of neuropeptide Y and [Pro^{34}]peptide YY (Table 1). In addition, the neuropeptide Y Y_5 receptor has high affinity for human pancreatic polypeptide, but much lower affinity for rat pancreatic polypeptide (Table 1). Neuropeptide Y Y_5 receptor mRNA is discretely localized in rat and human brain, primarily in piriform cortex, olfactory tubercle and hypothalamus (Nichol et al., 1999; Parker and Herzog, 1999). Neuropeptide Y Y_5 receptor binding sites have also been detected in these regions, although some groups fail to detect neuropeptide Y Y_5 receptor binding in hypothalamus (Dumont et al., 1998; Statnick et al., 1998; Widdowson et al., 1997a). Interestingly, neuropeptide Y Y_5 receptor mRNA is almost always localized in neurons that also express neuropeptide Y Y_1 receptor mRNA (Naveilhan et al., 1998). In the periphery, neuropeptide Y Y_5 receptor mRNA has been detected in rodent testis, spleen, pancreas, gastro-intestinal tract, vascular smooth muscle cells and cardiomyocytes (Gerald et al., 1996; Pellieux et al., 2000; Statnick et al., 1998). Neuropeptide Y has also been reported to alter diuresis, natriuresis and plasma glucose levels via neuropeptide Y Y_5 receptor activation in rats (Bischoff and Michel, 1999).

2.6. The neuropeptide Y y_6 receptor

The neuropeptide Y y_6 receptor was also initially identified by molecular cloning (Weinberg et al., 1996). A functional neuropeptide Y y_6 receptor gene is found in mice and rabbits, but a neuropeptide Y y_6 receptor gene has not been detected in rats (Burkhoff et al., 1998). The neuropeptide Y y_6 receptor gene is a non-functional pseudogene in rabbits and primates (Burkhoff et al., 1998). The neuropeptide Y y_6 receptor has a pharmacological profile that is similar to that of the neuropeptide Y Y_1 receptor, but is somewhat more tolerant of N-terminal truncation (Table 1). In mice, neuropeptide Y y_6 receptor mRNA has been detected in kidney, testis and brain, particularly in the hypothalamus (Burkhoff et al., 1998; Weinberg et al., 1996). Although the neuropeptide Y y_6 receptor clearly does not contribute to the physiological effects of neuropeptide Y in humans, this receptor must be taken into account when considering physiological effects of neuropeptide Y in mice.

2.7. Peptide YY-preferring receptor

A putative neuropeptide Y receptor known as the peptide YY-preferring receptor has been characterized in several tissues. This receptor has approximately 5- to 10-fold higher affinity for peptide YY than for neuropeptide Y and was initially found in crypt cells in the epithelium of the rat small intestine (Laburthe et al., 1986). Recently, Goumain et al. (2001) provided convincing evidence that the peptide YY-preferring receptor in rat small intestine is in fact the neuropeptide Y Y_2 receptor. Thus, the peptide YY-preferring receptor can now be equated with the neuropeptide Y Y_2 receptor.

3. Physiological role of neuropeptide Y in energy homeostasis

3.1. Effect of exogenous neuropeptide Y on energy intake and energy expenditure

Neuropeptide Y has potent effects on a variety of behavioral, physiological and endocrine systems that are critical in the modulation of energy homeostasis. Neuropeptide Y is the most potent orexigenic peptide identified to date. Nearly two decades ago, it was discovered that administration of exogenous neuropeptide Y directly to the brains of rats causes a tremendous increase in food consumption, even under conditions of satiation (Clark et al., 1984; Stanley and Leibowitz, 1984). Injection of neuropeptide Y directly into specific brain nuclei shows that the peptide is most effective in stimulating feeding when administered in the perifornical hypothalamus (Stanley et al., 1993). Hence, neuropeptide Y-containing neuronal pathways in the hypothalamus are most critical in the regulation of energy homeostasis. Central administration of neuropeptide Y to rats also decreases energy expenditure by decreasing sympathetic nervous system activity; as a result, thermogenic activity in brown adipose tissue, a key regulator of energy expenditure in rodents, is diminished (Egawa et al., 1991). By increasing food consumption and decreasing energy expenditure, central administration of neuropeptide Y results in a state of positive energy balance that will promote weight gain if chronically maintained. Central administration of neuropeptide Y also induces hyperinsulinemia, hyperglucagonemia, increased plasma non-esterified fatty acids and insulin resist-

ance. These effects are independent of the hyperphagic effect of neuropeptide Y (Marks and Waite, 1996).

Chronic central administration of neuropeptide Y to normal rats results in many of the physiological abnormalities observed in the obese state, including hyperphagia, accelerated weight gain, increased adiposity, hypertriglyceridemia, hyperinsulinemia and hypercorticosteronemia (Vettor et al., 1994; Zarjevski et al., 1993). As observed with other obesity models, neuropeptide Y-induced obesity is glucocorticoid-dependent, as adrenalectomy prior to chronic central neuropeptide Y administration prevents this obesity syndrome from developing (Sainsbury et al., 1997), and dexamethasone treatment of adrenalectomized rats restores the response to chronic neuropeptide Y administration (Zakrzewska et al., 1999). Hence, chronic central administration of neuropeptide Y results in obesity that is strikingly reminiscent of spontaneous, genetic and diet-induced obesity in rodents and man. Transgenic mice modestly overexpressing neuropeptide Y have significantly increased body weight, plasma glucose and plasma insulin compared to wild type mice when maintained on a palatable high sucrose diet, although not when maintained on a low fat diet (Kaga et al., 2001). This effect could be partially explained by significant but transient hyperphagia immediately following introduction of the high sucrose diet.

3.2. Role of endogenous neuropeptide Y in the modulation of energy homeostasis

A large body of evidence also suggests that endogenous neuropeptide Y plays a central role in energy homeostasis. Immunoneutralization of hypothalamic neuropeptide Y decreases feeding, even in rats deprived of food for 24 h (Shibasaki et al., 1993). Administration of neuropeptide Y antisense oligodeoxynucleotides to the brains of rats leads to the expected decrease in neuropeptide Y levels in the arcuate nucleus and also significantly reduces natural feeding behavior (Akabayashi et al., 1994; but see also Flynn et al., 1999). Furthermore, administration of certain potent neuropeptide Y receptor antagonists to rodents decreases food intake and body weight (see below).

Consistent with a role in the tonic modulation of energy homeostasis, expression of neuropeptide Y mRNA and the synthesis of neuropeptide Y are altered with changes in nutritional state and metabolic need, as well as in a number of genetic and diet-induced models of obesity. Depriving lean rats of food for 48 h leads to a significant increase in neuropeptide Y mRNA expression in the arcuate nucleus, and an increase in neuropeptide Y itself in the arcuate nucleus and the paraventricular nucleus (Sahu et al., 1988). Thus, increased neuropeptide Y synthesis and release may mediate the hyperphagic response observed after fasting. Streptozotocin-induced diabetic rats, a model of insulin-dependent diabetes with high metabolic demand, have increased neuropeptide Y mRNA expression in the arcuate nucleus and increased neuropeptide Y levels and neuropeptide Y release in the paraventricular nucleus, which may in part be responsible for the hyperphagia exhibited by these animals (Sahu et al., 1990). The obese *ob/ob* mouse, which lacks the hormone leptin, and the obese *db/db* mouse and Zucker rat, which do not have functional leptin receptors, both exhibit increased levels of hypothalamic neuropeptide Y mRNA and peptide (Sanacora et al., 1990; Stephens et al., 1995). Thus, increased neuropeptide Y transmission may be partially responsible for the hyperphagia and obesity that are characteristic of these mice (see below). A number of investigators have also assessed changes in the neuropeptide Y system in diet-induced obese rodents, but no single conclusion can be made from these studies, perhaps due to the myriad of differences between experiments. Suffice it to note that there appears to be a dysregulation of the neuropeptide Y system in animals that are prone to develop obesity under the appropriate dietary conditions (e.g., Bergen et al., 1999; Guan et al., 1998; Levin and Dunn-Meynell, 1997; Wilding et al., 1992).

Considering the wealth of data indicating that the neuropeptide Y system is important in energy homeostasis, results from transgenic mice in which the neuropeptide Y system has been manipulated have been somewhat paradoxical. Mice that are deficient in neuropeptide Y have normal food intake and body weight, and display the same hyperphagia as their wild type counterparts after food deprivation (Erickson et al., 1996a). Further studies indicated that plasma corticosterone, insulin, and glucose levels were normal in neuropeptide Y-deficient mice (Erickson et al., 1997). A lack of neuropeptide Y also did not affect the development of obesity induced by diet or chemical means (Hollopeter et al., 1998). Studies by a separate group, however, demonstrated that neuropeptide Y-deficient mice have decreased food intake after a 24–48 h fast (Bannon et al., 2000). The conflicting data on the effect of neuropeptide Y deficiency on energy homeostasis in mice is puzzling in view of the wealth of data supporting a role for the peptide in energy homeostasis. However, the results may suggest that mice readily compensate during development for the absence of a key neurotransmitter. Indeed, neuropeptide Y deficient mice are more sensitive to the hyperphagic effect of agouti-related protein, a melanocortin receptor antagonist that is co-expressed with neuropeptide Y in arcuate neurons (see below; Marsh et al., 1999b). Furthermore, many neurotransmitter and neuropeptide knockout mice do not display overt phenotypes; phenotypes are frequently seen only under specific physiological conditions or when the organism is stressed in some way. In this regard, it is interesting to note that the obese phenotype of *ob/ob* mice is attenuated when neuropeptide Y is absent, suggesting that neuropeptide Y is partially responsible for this obesity syndrome (Erickson et al., 1996b). These data also implicate neuropeptide Y as a downstream mediator of leptin action in the brain (see below). In contrast to the *ob/ob* mouse, the obesity characteristic of the genetically obese UCP-DTA or A^y mice was not affected when these mice were crossed with neuropeptide Y-deficient mice (Hollopeter et al., 1998).

3.3. Integration of neuropeptide Y with other brain pathways regulating energy homeostasis

In addition to neuropeptide Y, many other neurotransmitter and neuropeptide systems in the brain have been shown to influence energy homeostasis (for a review, see Spiegelman and Flier, 2001). Clearly, these manifold systems must interact with one another and the outputs of each of these systems must be highly integrated to achieve tight control of energy homeostasis. Consistent with its key role in the regulation of energy homeostasis, neuropeptide Y has been shown to interact with numerous neurotransmitter and neuropeptide systems that are thought to play a role in this process.

Leptin, a hormone secreted by adipose tissue that is critical in the regulation of body weight, exerts many of its physiological effects on body weight regulation by acting on target neurons in the brain (Spiegelman and Flier, 2001). It is likely that leptin is a key messenger that communicates information about adipose tissue energy stores to the central nervous system. Neuropeptide Y-containing neurons in the arcuate nucleus have been shown to express leptin receptors and, indeed, leptin has been shown to decrease neuropeptide Y mRNA levels in vivo and to decrease neuropeptide Y release from hypothalamic slices in vitro (Stephens et al., 1995). Thus, neuropeptide Y is probably one of the downstream mediators of leptin action in the brain. This conclusion is supported by the observation that the obese phenotype of the leptin-deficient *ob/ob* mouse is partially ameliorated by genetic deletion of neuropeptide Y (see above; Erickson et al., 1996b).

Activation of the melanocortin MC_3 and MC_4 receptors by melanocortin peptides such as α-melanocyte-stimulating hormone (α-MSH) decreases food intake, increases energy expenditure and decreases body weight (Vergoni and Bertolini, 2000). Conversely, blockade of these receptors by an endogenous antagonist known as agouti-related peptide (AGRP) has the opposite effect (Dinulescu and Cone, 2000). Furthermore, genetic ablation of the melanocortin MC_3 or MC_4 receptor has been shown to cause obesity in both mice and, in the case of the melanocortin MC_4 receptor, in humans (Spiegelman and Flier, 2001). Interestingly, AGRP is expressed exclusively in neuropeptide Y-containing neurons originating in the arcuate nucleus (Hahn et al., 1998). Thus, activation of arcuate neuropeptide Y neurons releases neuropeptide Y and AGRP, both of which increase food intake and body weight via activation of two distinct pathways. The obvious implication of this finding is that both arcuate neuropeptide Y/AGRP-containing neurons and arcuate melanocortin-containing neurons project to one or more common target neurons that express both neuropeptide Y receptors (Y_1 and/or Y_5; see below) and melanocortin receptors (MC_3 and/or MC_4). Since most arcuate melanocortin-containing neurons also express cocaine and amphetamine-regulated transcript (CART), another peptide that has been implicated in the central regulation of energy homeostasis (Elias et al., 1998), these target neurons probably also express CART receptors. Identification of the neurotransmitters employed by these target neurons may unveil a ''final common pathway'' in the regulation of energy homeostasis and would thus suggest very interesting additional basic research and drug development approaches in the field of obesity. It has been suggested that these target neurons contain γ-aminobutyric acid (GABA), CART, thyrotropin-releasing hormone (TRH), and/or melanin concentrating hormone (MCH), but more work is required to confirm this (Spiegelman and Flier, 2001).

Neuropeptide Y has also been shown to interact with a number of other hormones, neurotransmitters and neuropeptides that are thought to play a role in the regulation of body weight (e.g., insulin, galanin, GABA, corticotropin-releasing hormone, melanin concentrating hormone, orexin, ghrelin, biogenic amines, opiate peptides) (Kalra et al., 1999; Spiegelman and Flier, 2001). Neuropeptide Y may also be involved in metabolic control of energy homeostasis as evidenced by the decrease in neuropeptide Y levels and the decrease in food intake seen after inhibition of fatty acid synthase (Loftus et al., 2000). Taken together, these data strongly support a role for neuropeptide Y in the complex and highly integrated brain neurochemical system that regulates energy homeostasis.

3.4. Role of neuropeptide Y receptor subtypes in the regulation of energy homeostasis

To design potential anti-obesity drugs, it is necessary to identify the neuropeptide Y receptor subtypes that mediate the ability of neuropeptide Y to promote positive energy balance and increased body weight. Several lines of experimental evidence now point to a primary role of the neuropeptide Y Y_1 and Y_5 receptors in mediating the effects of neuropeptide Y on energy homeostasis. Central administration of selective neuropeptide Y Y_1 or Y_5 receptor peptide agonists to rodents increases food intake, suggesting that activation of either neuropeptide Y receptor subtype results in an orexigenic response (Cabrele et al., 2000; Gerald et al., 1996; Mullins et al., 2001; Parker et al., 2000). Conversely, administration of selective neuropeptide Y Y_1 and Y_5 receptor antagonists partially decreases spontaneous, neuropeptide Y-induced and/or fasting-induced food intake (see below). Administration of neuropeptide Y Y_5 receptor antisense oligonucleotides also results in a decrease in food intake (Schaffhauser et al., 1997; Tang-Christensen et al., 1998). Furthermore, the pharmacology of the neuropeptide Y receptor mediating neuropeptide Y-induced feeding and decreases in body temperature and energy expenditure in rats strongly resembles that of the neuropeptide Y Y_5 receptor (Hwa et al., 1999). Both the neuropeptide Y Y_1 and Y_5 receptors are also regulated by changes in nutritional status (Widdowson et al., 1997b; Zammaretti et al., 2001). The neuropeptide Y Y_1 and Y_5 receptors have also been proposed to play a role in the central and peripheral effects of neuro-

peptide Y on plasma glucose and insulin levels (Bischoff and Michel, 1999; Burcelin et al., 2001).

Studies of mice lacking neuropeptide Y Y_1 receptors also support a role for this receptor in mediating the effects of neuropeptide Y on energy homeostasis. Neuropeptide Y Y_1 receptor-deficient mice exhibit slightly diminished natural and neuropeptide Y-stimulated feeding. In addition, feeding following a 24-h fast was significantly diminished by about 45% (Kushi et al., 1998; Pedrazzini et al., 1998). Paradoxically, neuropeptide Y Y_1 receptor-deficient mice develop mild late onset obesity and moderate hyperinsulinemia. These mice exhibit reduced locomotor activity during the nocturnal period, which could be one mechanism by which the late onset obesity occurs.

Studies of mice lacking neuropeptide Y Y_5 receptors have failed to provide strong support for a role of this receptor in mediating the effects of neuropeptide Y on energy homeostasis (Marsh et al., 1998). Neuropeptide Y Y_5 receptor-deficient mice have normal growth and feeding when young. Core body temperature in neuropeptide Y Y_5 receptor-deficient mice is normal, indicating a lack of a gross effect on metabolic rate. Feeding induced by low doses of neuropeptide Y was unchanged, while feeding induced by higher doses of neuropeptide Y was reduced in neuropeptide Y Y_5 receptor-deficient mice. Interestingly, neuropeptide Y-induced feeding was completely abolished in neuropeptide Y Y_5 receptor-deficient mice in which neuropeptide Y Y_1 receptors were also blocked by a neuropeptide Y Y_1 receptor antagonist (Marsh et al., 1998). This confirms that activation of both neuropeptide Y Y_1 and Y_5 receptors are responsible for neuropeptide Y-induced feeding. Neuropeptide Y Y_5-deficient *ob/ob* mice were equally as obese as *ob/ob* mice, indicating that the attenuation of obesity in the *ob/ob* mouse lacking neuropeptide Y is not mediated through the Y_5 receptor (Marsh et al., 1998). Like the neuropeptide Y Y_1 receptor-deficient mice, neuropeptide Y Y_5 receptor-deficient mice unexpectedly develop a mild late onset obesity; however, this is more likely due to an increase in food intake.

The data from the neuropeptide Y Y_1 and Y_5 receptor-deficient mice confirm that both receptors mediate neuropeptide Y-induced food intake and that the neuropeptide Y Y_1 receptor may also play a minor role in spontaneous food intake and a more significant role in food intake after deprivation. As discussed above, it is possible that these data underestimate the role of the neuropeptide Y Y_1 and Y_5 receptors in normal energy homeostasis if mice can readily compensate for their absence. For example, an increased involvement of the neuropeptide Y y_6 receptor in energy homeostasis may occur in the absence of the neuropeptide Y Y_1 or Y_5 receptor in mice. It is also possible that the neuropeptide Y Y_1 and Y_5 receptors are only involved in energy homeostasis under specific conditions (e.g., situations of negative energy balance such as starvation, obese patients who have lost substantial weight, etc.) It would also be interesting to examine energy homeostasis in mice lacking both the neuropeptide Y Y_1 and Y_5 receptors. Unfortunately, the close proximity of the genes for the neuropeptide Y Y_1 and Y_5 receptors precludes the generation of mice lacking both receptors via simple cross breeding of the individual knockout mice.

The selective neuropeptide Y Y_2 receptor agonist C2-neuropeptide Y does not increase food intake, suggesting that the receptor does not mediate the effects of neuropeptide Y on energy homeostasis (Gerald et al., 1996). However, mice deficient for the neuropeptide Y Y_2 receptor subtype have increased body weight, food intake and fat deposition. Regulation of feeding and body weight is normal upon refeeding after starvation. Neuropeptide Y Y_2 receptor-deficient mice maintain the feeding response elicited by neuropeptide Y, but have an attenuated response to leptin administration (Naveilhan et al., 1999). These data suggest a possible role for the neuropeptide Y Y_2 receptor in normal energy homeostasis and in the response to leptin. The effect of the neuropeptide Y Y_2 receptor may be indirect, due to effects of neuropeptide Y Y_2 receptor activation on the release of neuropeptide Y and other neurotransmitters (see above). The recent disclosure of a non-peptide neuropeptide Y Y_2 receptor antagonist, BIIE0246 (Doods et al., 1999), may provide a tool for further testing the role of the neuropeptide Y Y_2 receptor in the regulation of energy homeostasis.

The selective neuropeptide Y Y_4 receptor agonist rat pancreatic polypeptide does not increase food intake, suggesting that activation of the neuropeptide Y Y_4 receptor is not responsible for mediating the effects of neuropeptide Y on energy homeostasis (Gerald et al., 1996). Herzog et al. (2001) have recently generated neuropeptide Y Y_4 receptor knockout mice. These mice tend to be leaner than their wild type counterparts, although there was little or no difference in food intake, adipose tissue mass or serum levels of insulin, glucose, leptin and corticosterone. Interestingly, *ob/ob* mice lacking the neuropeptide Y Y_4 receptor had similar body weight, increased food intake, less fat mass, decreased liver weight, decreased liver lipid content and improved fertility relative to control *ob/ob* mice. These data may suggest a role for the neuropeptide Y Y_4 receptor in energy homeostasis, but further studies are required, particularly since obesity or leanness are proving to be common phenotypes of many knockout mice.

Overall, the data suggest a primary, direct role for the neuropeptide Y Y_1 and Y_5 receptors in mediating the effects of neuropeptide Y on energy homeostasis. The neuropeptide Y Y_2 and/or Y_4 receptors may play a subtle, perhaps indirect role in energy homeostasis, but further work is required to confirm this.

4. Development of non-peptide neuropeptide Y receptor antagonists as therapeutic agents for the treatment of obesity

Within the pharmaceutical industry, there has been great interest in the development of neuropeptide Y Y_1 and Y_5

receptor antagonists as potential drugs for obesity management. There is now a significant body of primary and patent literature describing potent and selective neuropeptide Y Y_1 receptor antagonists, and an even larger body of literature devoted to neuropeptide Y Y_5 receptor antagonists. The following discussion focuses on the pharmacology of non-peptidic neuropeptide Y receptor antagonists, with emphasis on reports from the primary literature that attempt to correlate neuropeptide Y receptor subtype-selective antagonism with effects on food intake and body weight. Several excellent reviews provide a comprehensive discussion of neuropeptide Y receptor antagonists that have been disclosed (Carpino, 2000; Hammond, 2001; Ling, 1999; Wieland et al., 2000; Zimanyi and Poindexter, 2000).

4.1. Neuropeptide Y Y_1 receptor antagonists

The discovery of potent and selective neuropeptide Y Y_1 receptor antagonists with oral bioavailability and central nervous system (CNS) penetrability has proved to be a challenging endeavor. Initially reported neuropeptide Y Y_1 receptor antagonists failed to cross the blood–brain barrier, and only recently have orally available brain penetrant neuropeptide Y Y_1 receptor antagonists been developed.

The first potent and selective non-peptidic neuropeptide Y Y_1 receptor antagonist, BIBP3226 (Rudolf et al., 1994), was designed to mimic the C-terminal region of neuropeptide Y (Fig. 1; Grundemar and Bloom, 1997). Structure–activity relationship studies confirmed that correct spatial arrangement with respect to the guanidine, phenol, and diphenylacetamide are critical for nanomolar affinity of BIBP3226 at the neuropeptide Y Y_1 receptor (Grundemar and Bloom, 1997). Initially, it was shown that BIBP3226 has high affinity for human and rat neuropeptide Y Y_1 receptors (human Y_1 K_i=5.1 nM, rat Y_1 K_i=6.8 nM) and is inactive at neuropeptide Y Y_2 receptors (K_i>1000 nM) (Rudolf et al., 1994; Wieland et al., 1995). It was subsequently shown that BIBP3226 does not have significant affinity for neuropeptide Y Y_4 and Y_5 receptors (Gerald et al., 1996). BIBP3226 is perhaps the most widely studied neuropeptide Y receptor antagonist, and its use as a tool to investigate the vascular pharmacology of neuropeptide Y mediated by the neuropeptide Y Y_1 receptor has been reported (Doods et al., 1996).

Since BIBP3226 does not penetrate the blood–brain barrier (Doods et al., 1996), central administration of the compound is necessary to study the role of CNS neuropeptide Y Y_1 receptors in the regulation of food intake and energy expenditure. Administration of BIBP3226 intracerebroventricularly (Kask et al., 1998; O'Shea et al., 1997) or into the paraventricular nucleus (Morgan et al., 1998) was reported to block neuropeptide Y-induced food intake. However, observations consistent with CNS toxicity were seen on administration of BIBP3226 by these routes and these observations have cast doubt on the specificity of these effects (Doods et al., 1996; Morgan et al., 1998).

A structurally related analogue, BIBO3304, is claimed to cause less CNS toxicity than BIBP3226 (Fig. 1; Wieland et al., 1998). This compound has high affinity for cloned rat and human neuropeptide Y Y_1 receptors (rat Y_1 IC_{50}=0.72 nM, human IC_{50}=0.38 nM), and no significant affinity for the other neuropeptide Y receptor subtypes (IC_{50}>10.000 nM). In cells expressing the neuropeptide Y Y_1 receptor,

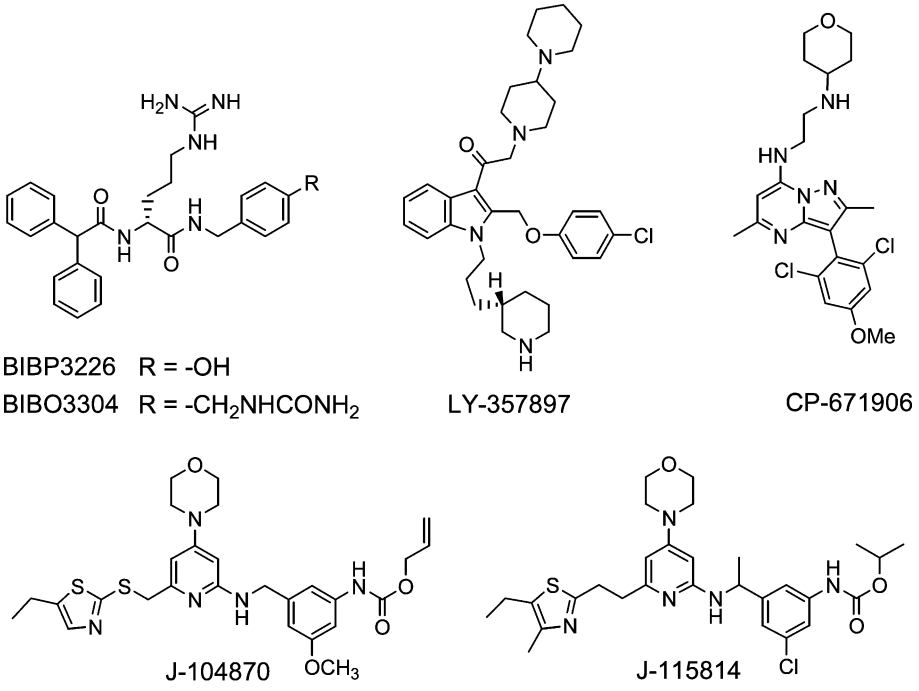

Fig. 1. Structures of neuropeptide Y Y_1 receptor antagonists.

BIBO3304 blocked neuropeptide Y-induced inhibition of forskolin-stimulated cAMP synthesis (pK_b=9.1), confirming that the compound is an antagonist. Central administration of BIBO3304 to rats attenuated feeding induced by neuropeptide Y and by food deprivation. There was no effect of BIBO3304 on feeding induced by noradrenaline or galanin and the inactive enantiomer of BIBO3304 (Y_1 K_i>1000 nM) did not inhibit neuropeptide Y-elicited food intake or feeding after food deprivation. These data suggest that the effects of BIBO3304 on food intake were mediated specifically by blockade of the neuropeptide Y Y_1 receptor (Wieland et al., 1998). In addition, intracerebroventricular administration of BIBO3304 to Zucker and Wistar rats for 1 week decreased body weight gain (Doods et al., 1997). These data indicate that activation of the neuropeptide Y Y_1 receptor promotes food intake and body weight gain under physiological and pathophysiological conditions, and that neuropeptide Y Y_1 receptor antagonists may be useful anti-obesity agents.

A group from Eli Lilly reported the first neuropeptide Y Y_1 receptor antagonist with sub-nanomolar potency, the substituted indole derivative LY-357897 (Fig. 1; Hipskind et al., 1997). The discovery of LY-357897 was achieved by a classical medicinal chemistry approach from a screening lead with weak affinity for the neuropeptide Y Y_1 receptor. An important conceptual step in the discovery of LY-357897 was appendage of a basic group to the indole nitrogen to improve potency. This strategy was adopted due to the importance of the dibasic functionality of the C-terminal region of neuropeptide Y for neuropeptide Y binding to the neuropeptide Y Y_1 receptor.

LY-357897 displayed a K_i of 0.75 nM at cloned human neuropeptide Y Y_1 receptors, and no affinity at cloned human neuropeptide Y Y_2, Y_4, and Y_5 receptors (K_i>10 μM). The compound antagonized neuropeptide Y-induced Ca^{2+} mobilization (IC_{50}=3.2 nM) and neuropeptide Y-induced inhibition of forskolin-stimulated cAMP synthesis in cells expressing the human neuropeptide Y Y_1 receptor (IC_{50}=1.8 nM). Unfortunately, the compound could not be evaluated systemically due to a lack of oral and subcutaneous bioavailability. However, LY-357897 administered into the lateral ventricle of mice dose-dependently blocked food consumption elicited by intracerebroventricularly administered neuropeptide Y (ED_{50}=17 nmol). It was reported that at doses of LY-357897 that blocked neuropeptide Y-induced food intake, no changes in motor function or behavioral effects were observed, consistent with a neuropeptide Y Y1 receptor-specific mechanism for the effect.

Banyu workers have reported studies with the potent and selective neuropeptide Y Y_1 receptor antagonists J-104870 and J-115814 from a diaminopyridine series (Fig. 1; Kanatani et al., 1999, 2001). J-104870 is the first reported potent, orally bioavailable, brain penetrant neuropeptide Y Y_1 receptor antagonist (Kanatani et al., 1999). The compound has high affinity for cloned rat and human neuropeptide Y Y_1 receptors (human Y_1 K_i=0.26 nM, rat Y_1 K_i=0.51 nM) and no significant affinity for human neuropeptide Y Y_2, Y_4, and Y_5 receptors (K_i>1000 nM). J-104870 dose-dependently inhibited the neuropeptide Y-induced increase in intracellular Ca^{2+} levels in cells expressing human neuropeptide Y Y_1 receptors (IC_{50}=3.2 nM), confirming that it is an antagonist. Oral administration of J-104870 (100 mg/kg) to Zucker rats suppressed spontaneous feeding over 24 h by 18%, affording brain levels of 0.5 μM 24 h after dosing. A similar reduction in spontaneous food intake was observed on intracerebroventricular injection of J-104870 to Zucker rats. In contrast, J-104870 had no effect on spontaneous food intake when administered intracerebroventricularly to satiated Sprague–Dawley rats, although the compound did block neuropeptide Y-induced food intake. These data support the conclusion that elevated neuropeptide Y levels present in the brains of Zucker rats are partially responsible for the obese phenotype of these rats.

The related compound J-115814 showed high affinity for human, rat and mouse neuropeptide Y Y_1 receptors (human Y_1 K_i=1.4 nM; rat Y_1 K_i=1.8 nM; mouse Y_1 K_i=1.9 nM). Much lower affinity was seen at human neuropeptide Y Y_2 (K_i>10,000 nM), Y_4 (K_i=620 nM) and Y_5 (K_i=6000 nM) receptors, as well as mouse neuropeptide Y y_6 receptors (K_i>10,000 nM) (Kanatani et al., 2001). Furthermore, the compound had no significant activity at 50 other receptors tested. The antagonist character of J-115814 was confirmed by its inhibition of neuropeptide Y-induced increases in intracellular Ca^{2+} levels in cells expressing human neuropeptide Y Y_1 receptors (IC_{50}=6.8 nM). Administration of J-115814 intravenously or intracerebroventricularly to satiated rats dose responsively suppressed neuropeptide Y-induced food intake by up to 50%. There was no effect on food intake, nor were any behavioral changes observed when J-115814 was administered alone.

When J-115814 was administered intraperitoneally to wild type or neuropeptide Y Y_5 receptor-deficient mice, neuropeptide Y-induced food intake was inhibited by about 50%. However, J-115814 failed to suppress neuropeptide Y-elicited food intake in Y_1 receptor-deficient mice, supporting the notion that J-115814 inhibits neuropeptide Y-induced food intake by a neuropeptide Y Y_1 receptor-specific mechanism. When administered intraperitoneally to *db/db* mice or to C57BL6 mice, J-115814 caused a reduction in spontaneous feeding with a minimum effective dose of 10 mg/kg. Hypothalamic levels of J-115814 were consistent with the effects on food intake being mediated by neuropeptide Y Y_1 receptor blockade. The greater inhibition of food intake seen in *db/db* mice at the minimum effective dose is consistent with the proposal that increased activation of neuropeptide Y Y_1 receptors plays an important role in pathophysiological hyperphagia and obesity in *db/db* mice (Kanatani et al., 2001).

Recently, a group from Pfizer and Neurogen has also reported an orally bioavailable neuropeptide Y Y_1 receptor antagonist that crosses the blood–brain barrier, CP-671,906 (Fig. 1; Griffith et al., 2001). The compound showed high affinity for cloned rat and human neuropeptide Y Y_1 recep-

tors (rat Y_1 K_i=3.5 nM, human Y_1 K_i=1.5 nM), with no significant affinity for neuropeptide Y Y_2 and Y_5 receptors (K_i>1000 nM). In addition, CP-671,906 did not show significant activity at more than 35 other receptors, enzymes and ion channels. The functional antagonist activity of CP-671,906 was demonstrated by its inhibition of neuropeptide Y-induced intracellular Ca^{2+} flux in cells expressing neuropeptide Y Y_1 receptors (K_b=11 nM), as well as by its inhibition of neuropeptide Y-induced contractions of rabbit vas deferens (K_b=26 nM). In rats administered 40 mg/kg orally, CP-671,906 achieved plasma levels >1 μM and a brain to plasma ratio of 1. The compound blocked neuropeptide Y-induced food intake in rats when dosed 4 h prior to neuropeptide Y administration, which corresponded to the time at which brain interstitial fluid concentrations peaked at levels 10- to 70-fold over the K_i. Food intake elicited by the neuropeptide Y Y_5 receptor agonist bovine pancreatic polypeptide was not affected by CP-671,906, consistent with a neuropeptide Y Y_1 receptor-specific effect of the compound. CP-671,906 (40 mg/kg orally) inhibited refeeding in food-deprived Wistar rats at 2, 4, and 24 h after dosing, but did not affect dark-cycle food intake in free-feeding rats. CP-671,906 (10 mg/kg orally) also decreased food intake in Zucker rats by 15% over 2 days, but caused adverse effects at slightly higher doses (40 mg/kg orally). The compound also inhibited food intake in overweight beagle dogs, but behavioral changes were noted at the dose tested (10 mg/kg orally). Overall, it is difficult to rule out the possibility that the anorectic effects of CP-671,906 are due to a non-specific mechanism given that anorectic doses approach doses that produce adverse effects in several species.

In general, data with neuropeptide Y Y_1 receptor antagonists support a role for the neuropeptide Y Y_1 receptor in the modulation of energy homeostasis, particularly feeding induced by deprivation in normal rodents or feeding in genetically obese rodents. Orally active, brain penetrant neuropeptide Y Y_1 receptor antagonists have only recently been developed, but the compounds reported thus far are not very potent in vivo. It will likely be necessary to develop compounds with greater in vivo potency and/or a lower incidence of adverse effects.

4.2. Neuropeptide Y Y_5 receptor antagonists

The first potent, selective neuropeptide Y Y_5 receptor antagonist was the aminoquinazoline CGP 71683A reported in 1998 by a group from Novartis and Synaptic (Fig. 2; Criscione et al., 1998). Starting from a lead with weak

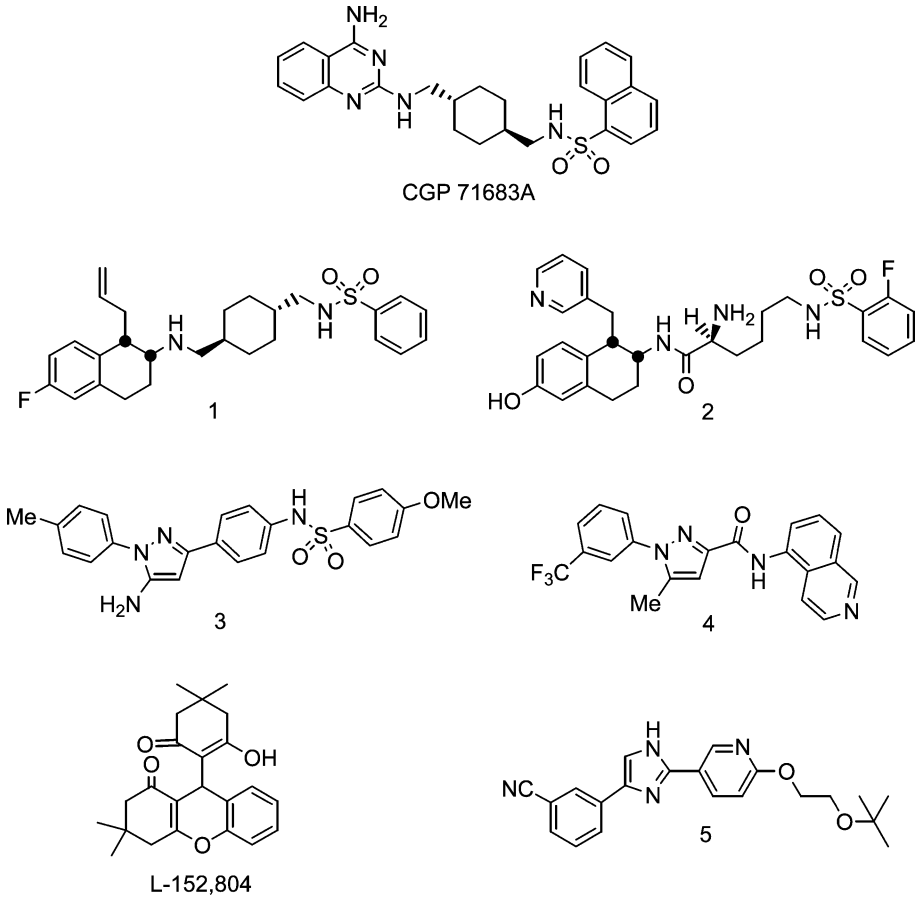

Fig. 2. Structures of neuropeptide Y Y_5 receptor antagonists.

affinity for neuropeptide Y Y_1 and Y_5 receptors, CGP 71683A was discovered through a process of combinatorial chemistry and traditional medicinal chemistry. Structure–activity relationship studies suggest that important pharmacophore elements of CGP 71683A are the hydrogen bond donor–acceptor properties of the aminoquinazoline ring and the hydrogen bond accepting sulfonamide separated by an appropriate distance by the hydrophobic spacer (Rueeger et al., 2000).

CGP 71683A has high affinity for the rat and human neuropeptide Y Y_5 receptors (rat Y_5 IC_{50}=1.4 nM; human Y_5 IC_{50}=2.9 nM) and low affinity for human neuropeptide Y Y_1, Y_2 and Y_4 receptors (IC_{50}>1000 nM). The compound was an antagonist of the rat neuropeptide Y Y_5 receptor, selectively inhibiting neuropeptide Y-induced intracellular Ca^{2+} transients in cells expressing neuropeptide Y Y_5 receptors. Intraperitoneal injection of CGP 71683A to satiated lean rats at a dose of 10 mg/kg inhibited neuropeptide Y-induced food intake by 50% and attenuated feeding after food deprivation, but had little effect on food intake in free-feeding rats. Rats that were chronically dosed with CGP 71683A showed reduced food intake and body weight gain over the first few days of treatment, but food intake and body weight gain subsequently approached control levels. A combination of intraperitoneally administered CGP 71683A and the neuropeptide Y Y_1 receptor antagonist BIBO3304 was recently reported to produce anorectic effects in lean rats, Zucker rats, and *ob/ob* mice at doses where either agent alone was ineffective (Duhault et al., 2000). CGP 71683A has recently been reported to have potent affinity for the 5-hydroxytryptamine (5-HT) reuptake recognition site and muscarinic receptors, so the possibility that CGP 71683A produces anorectic effects through mechanisms other than neuropeptide Y Y_5 receptor blockade cannot be ruled out (Della Zuana et al., 2001). Indeed, it was recently reported that the anorectic effects of CGP 71683A are identical in wild type and Y_5 receptor-deficient mice, thus indicating that the anorectic effects of the compound are non-specific (Criscione et al., 2001).

A novel series of neuropeptide Y Y_5 receptor antagonists based on a β-aminotetralin scaffold has been reported by a group at R.W. Johnson (Youngman et al., 2000). Potent analogues such as 1 and 2 were identified through optimization of a micromolar screening lead (Fig. 2). The latter compound contains the bis-(aminomethyl)cyclohexyl linker present in CGP71683A. Compounds 1 and 2 bind with high affinity to human neuropeptide Y Y_5 receptors (IC_{50}=23 and 1.0 nM, respectively), and inhibited peptide YY-induced [^{35}S]GTPγS binding to membranes from Bowes melanoma cells transfected with neuropeptide Y Y_5 receptors. Furthermore, 1 showed no significant affinity for human neuropeptide Y Y_1 and Y_2 receptors, as well as for over 30 G-protein coupled receptors and ion channels. Intraperitoneal administration of 1 (30 mg/kg) to fasted rats was reported to significantly reduce food consumption by 35% 6 h after dosing. While compounds in the series were said to be generally well-tolerated, no data addressing the specificity of the effects of 1 on food intake or correlating CNS exposure to drug with the anorectic effects were reported in this study.

The R.W. Johnson group has also described a structurally distinct pyrazole series represented by 3 and 4 (Fig. 2; Kordik et al., 2001a,b). Compounds 3 and 4 were reported to show IC_{50}s of 15 and 80 nM, respectively, against the human neuropeptide Y Y_5 receptor, and did not bind significantly to human neuropeptide Y Y_1 or Y_2 receptors. No data confirming the antagonistic properties of these compounds at neuropeptide Y Y_5 receptors was reported. At a dose of 30 mg/kg administered intraperitoneally to rats, 4 decreased fasting-induced feeding by 43% in the period 2–6 h after administration. Compound 4 was said to be well tolerated. However, in the absence of data to support neuropeptide Y Y_5 receptor specificity, the mechanism of the anorectic effects of 4, like 1, must be considered circumstantial and open to question.

L-152,804 is an orally bioavailable, brain-penetrant neuropeptide Y Y_5 receptor antagonist that has reasonable affinity for human and rat neuropeptide Y Y_5 receptors (human Y_5 K_i=26 nM; rat Y_5 K_i=31 nM) and low affinity for human neuropeptide Y Y_1, Y_2, and Y_4 receptors (K_i>10,000 nM) (Fig. 2; Kanatani et al., 2000). The antagonist activity of L-152,804 was confirmed in cells expressing the human neuropeptide Y Y_5 receptor, in which L-152,804 inhibited the neuropeptide Y-induced increase in intracellular Ca^{2+} levels (IC_{50}=210 nM). When administered intracerebroventricularly to satiated rats, L-152,804 inhibited food intake elicited by the neuropeptide Y Y_4/Y_5 receptor-selective agonist, bovine pancreatic polypeptide. Administration of L-152,804 alone had no effect on food intake, and was reported not to induce overt behavioral changes, indicating that the anorectic effect of the compound is neuropeptide Y Y_5 receptor-specific. When administered orally to rats, L-152,804 (10 mg/kg) also inhibited food intake elicited by intracerebroventricular bovine pancreatic polypeptide. However, L-152,804 administered intracerebroventricularly or orally failed to inhibit neuropeptide Y-stimulated food intake, despite brain levels of 2.9 μM 2 h after oral administration. Based on these observations, it was concluded that activation of the neuropeptide Y_5 receptor does not substantially contribute to food intake in rodents. However, further studies with functionally more potent neuropeptide Y Y_5 receptor antagonists would address this more definitively.

A group from Pfizer and Neurogen has recently reported extensive pharmacological evaluation of a more potent, orally bioavailable, brain penetrant neuropeptide Y Y_5 receptor antagonist, the 2,4-diarylimidazole 5, in models of feeding and energy expenditure (Fig. 2; Elliott et al., 2001). Compound 5 showed high affinity for human neuropeptide Y Y_5 receptors (IC_{50}=1.2 nM), and inhibited neuropeptide Y-induced Ca^{2+} mobilization in Bowes melanoma cells expressing the neuropeptide Y Y_5 receptor (IC_{50}=0.4 nM). The compound did not have significant affinity for human neuropeptide Y Y_1 or Y_2 receptors (IC_{50}>1000 nM),

or for over 50 other receptors. Food intake elicited by bovine pancreatic polypeptide was inhibited by 56% after oral administration of compound 5 (30 mg/kg). Brain and CSF levels were found to be 4 and 0.2 μM, respectively, 0.5 h after dosing. These data demonstrate that compound achieves excellent CNS exposure and can block a neuropeptide Y Y_5 receptor-specific effect in vivo. However, compound 5 at a dose of 40 mg/kg orally failed to inhibit feeding following food deprivation in rats, and had no effect on spontaneous feeding at a dose of 30 mg/kg orally. Thermogenic effects were not evident with compound 5, as it did not cause significant changes in oxygen consumption or respiratory quotient when dosed at 30 mg/kg orally. There was no effect of the compound on spontaneous locomotor activity, consistent with a lack of overt behavioral effects. These data indicate that while activation of the neuropeptide Y Y_5 receptor with an exogenous ligand can promote food intake, it does not play a significant role in regulation of food intake and energy expenditure in lean rats under physiological conditions.

4.3. Potential side effects of neuropeptide Y receptor antagonists

Because neuropeptide Y is involved in a wide variety of physiological processes, many of which are mediated via neuropeptide Y Y_1 and Y_5 receptors, it is possible that neuropeptide Y Y_1 and Y_5 receptor antagonists developed for the treatment of obesity will be associated with specific mechanism-based side effects. Neuropeptide Y Y_1 receptor activation is known to lead to increased blood pressure, proliferation of neuronal precursor cells, anxiolysis, analgesia, neurogenic inflammation and modulation of pituitary hormone secretion (Wahlestedt et al., 1993; Grundemar and Bloom, 1997; El Majdoubi et al., 2000; Hansel et al., 2001; Naveilhan et al., 2001). Interestingly, neuropeptide Y Y_1 receptor antagonists such as BIBP3226 have no effect on blood pressure, suggesting that hypotension will not be an issue with these drugs (Doods et al., 1996). There have been reports that neuropeptide Y Y_1 receptor antagonists are anxiogenic and accelerate the onset of puberty (Kask et al., 1996; Pralong et al., 2000). The latter effect presumably reflects effects of the neuropeptide Y Y_1 receptor antagonist on pituitary gonadotropin secretion. All studies reported to date used intracerebroventricular administration of neuropeptide Y Y_1 receptor antagonists and additional studies with newer, orally active, brain penetrant compounds would be more informative.

Similarly, neuropeptide Y Y_5 receptor activation has been associated with anti-epileptic effects, attenuation of opiate withdrawal, modulation of circadian rhythms, regulation of pituitary hormone secretion, natriuresis and decreases in plasma glucose (Bischoff and Michel, 1999; Marsh et al., 1999a; Raposinho et al., 1999; Yannielli and Harrington, 2001). Unfortunately, the effect of neuropeptide Y Y_5 receptor antagonists on these processes has not been reported.

5. Summary and perspective

Data from mice lacking neuropeptide Y or the neuropeptide Y_1 receptor suggest that activation of the neuropeptide Y Y_1 receptor by neuropeptide Y plays a role in maintaining normal food intake as well as food intake after deprivation. Studies with selective neuropeptide Y Y_1 receptor antagonists also suggest a role for neuropeptide Y Y_1 receptors in maintaining food intake under conditions of real or apparent deprivation (e.g., food-deprived animals and genetically obese animals in which leptin signaling to the hypothalamus has been disrupted). The role of the neuropeptide Y Y_5 receptor in energy homeostasis is less clear, however. Mice lacking the neuropeptide Y Y_5 receptor do not differ from wild type mice in paradigms that assess food intake and body weight under a variety of conditions. Furthermore, the reported effects of neuropeptide Y Y_5 receptor antagonists on food intake and body weight are conflicting. Although administration of some neuropeptide Y Y_5 receptor antagonists is associated with reduced food intake and body weight gain, evidence for the specificity of these effects is lacking.

The modest effects of neuropeptide Y deficiency, neuropeptide Y Y_1 receptor deficiency and neuropeptide Y Y_1 receptor antagonists and the lack of any consistent effect of neuropeptide Y Y_5 receptor deficiency and neuropeptide Y Y_5 receptor antagonists on energy homeostasis suggest that the role of neuropeptide Y in the regulation of body weight is more complicated than previously envisioned. Overall, these results may indicate that the high level of redundancy in the regulation of body weight insures that mice can substantially compensate for the loss of a single neuropeptide or neuropeptide receptor under normal conditions. However, the data obtained to date suggests that neuropeptide Y plays a critical role in energy homeostasis under very specific physiological conditions, particularly conditions of real or apparent deprivation. These data indicate that neuropeptide Y receptor antagonists may be most useful in human conditions where appetite is increased and energy expenditure is decreased due to activation of the starvation response. Such conditions include obese patients who are dieting, formerly obese patients who have lost substantial weight, and patients with complete or partial leptin deficiency.

Significant progress has been made in the identification of structurally diverse, orally bioavailable neuropeptide Y Y_1 and Y_5 receptor antagonists that cross the blood–brain barrier. However, there is a clear need for further studies with both neuropeptide Y Y_1 and Y_5 receptor antagonists in order to clarify their potential as anti-obesity agents. Most studies have been performed in lean rodents or in genetically obese rodents that do not mimic common obesity in the human population. It would be desirable to evaluate the effects of compounds in diet-induced obese rodents and non-rodents as disease models that more closely mimic human obesity. Other critical issues in the development of neuropeptide Y receptor antagonists as effective agents for

obesity management are the need to overcome counterbalancing effects of the multiple complementary mechanisms involved in energy homeostasis that tend to oppose any changes in body weight and the identification of patient subclasses most likely to benefit from treatment.

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