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Neuropeptide Y and energy homeostasis: insights from Y receptor knockout models

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Abstract

A complex system has evolved to regulate food intake and to maintain energy homeostasis. A series of short-term hormonal and neural signals that derive from the gastrointestinal tract, such as cholecystokinin (CCK), pancreatic polypeptide (PP) and peptide YY-(3–36), recently discovered to regulate meal size. Others such as ghrelin initiate meals, and insulin and leptin, together with circulating nutrients, indicate long-term energy stores. All these signals act on central nervous system sites which converge on the hypothalamus, an area that contains a large number of peptide and other neurotransmitters that influence food intake with neuropeptide Y (NPY) being one of the most prominent ones. Five Y receptors are known which mediate the action of neuropeptide Y and its two other family members, peptide YY and pancreatic polypeptide. Elevated neuropeptide Y expression in the hypothalamus leads to the development of obesity and its related phenotypes, Type II diabetes and cardiovascular disease. The limited availability of specific pharmacological tools and the considerable number of Y receptors have made it difficult to delineate their individual contributions to the regulation of energy homeostasis. However, recent studies analysing transgenic and knockout neuropeptide Y and Y receptor mouse models have started to unravel some of the individual functions of these Y receptors potentially also helping to develop novel therapeutics for a variety of physiological disorders including obesity.

Keywords: Neuropeptide Y; Obesity; Transgenic animal; Food intake; Satiety signal

1. Introduction

The prevalence of obesity in developed affluent countries is alarming and obesity is now a major contributor to morbidity and mortality, with fat deposition in the abdominal area being a particular risk factor for Type II diabetes and cardiovascular disease (Bakris et al., 1996; Ikeda et al., 1999; Seidell, 1999). A large number of neurotransmitters and hormones and a variety of different neuronal pathways have been shown to play a role in the regulation of energy homeostasis and body weight (Kalra et al., 1999). The major centers in the central nervous system where the regulation takes place are the hypothalamus and certain brain stem nuclei (Blevins et al., 2002). The majority of the known hormones and peptides that regulate energy homeostasis exert an important component of their effects via actions on the neuropeptide Y (NPY) system (Erickson et al., 1996b; Kask et al., 2000; Zakrzewska et al., 1999), demonstrating the pivotal role of neuropeptide Y in coordinating this important physiological process.

Neuropeptide Y, in addition to the strong stimulatory effect on food consumption, also regulates blood pressure, induces anxiolysis, enhances memory retention, affects circadian rhythms as well as modulates hormone release (Hokfelt et al., 1998). Two other closely related family members peptide YY (PYY) and pancreatic polypeptide (PP) produced by L type cells in the small intestine and colon or in F type cells in the pancreas, respectively, act mainly as hormones in an endo- and exocrine fashion (Hazelwood, 1993). However, they can also access specific Y receptors in the hypothalamus and the brain stem thereby regulating pancreatic and gastric secretion as well as gastric and intestinal motility via modulation of vagal output further highlighting the importance of the whole neuropeptide Y system for regulating energy homeostasis.

Neuropeptide Y mediates its effects through the activation of at least five different receptors: Y_1, Y_2, Y_4, Y_5, and in mouse also y_6 (Blomqvist and Herzog, 1997; Naveilhan et al., 1998; Parker and Herzog, 1999), all acting in an

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Table 1
Transgenic animal models of neuropepeptide Y family members

	Ligand transgenic models			Ligand knockout models			
	NPY (rat)	NPY (mouse)	PP (mouse)	NPY	NPY/AgRP	NPY/ob	NPY/Ay
Food intake	◄►	◄►	▼	◄►	◄►	▼	◄►
Body weight	◄►	◄►	▼	◄►	◄►	▼	◄►
Adiposity	◄►	◄►	▼	◄►	◄►	▼	◄►
Heart rate	◄►						
Blood pressure	◄►						
Anxiety	▼	◄►		▲			
Nociception				▲			
Bone mass							
Memory	▼						
Fertility				◄►		▲	
Alcohol consumption		▼		▲			
Seizure susceptibility	◄►			▲			
Neurogenesis				▼			
References	Michalkiewicz and Michalkiewicz, 2000; Vezzani et al., 2002	Thiele et al., 2000; Inui et al., 1998	Ueno et al., 1999	Thiele et al., 2000; Erickson et al., 1996a,b; Bannon et al., 2000	Qian et al., 2002	Erickson et al., 1996a,b	Hollopeter et al., 1998

▲ = increase; ◄► = unaltered; ▼ = decreased.

inhibitory fashion. neuropeptide Y and peptide YY have identical affinity for all Y receptors, with pancreatic polypeptide preferring the Y_4 receptor.

Delineating the function of the neuropeptide Y system, in the past, was mainly relying on the use of pharmacological approaches employing modified peptide ligands and a few synthetic molecules. Interpretation of these results was difficult due to the poor knowledge of the in vivo selectivity and the distinct metabolic or pharmacokinetic properties of the compounds used. It is difficult to ensure that ligands that are highly selective in an in vitro system (e.g. binding properties on brain tissue or recombinant receptor preparations) do not cross-react with other known or yet unidentified receptors in vivo. In addition, their action can vary strongly depending on the dose, the site and the mode of administration (acute versus chronic) of the compound. Many questions regarding the functional contributions of the different Y receptors to a particular physiological process such as the regulation of energy balance have been left unanswered.

Table 2
Single Y receptor knockout models

	Y1	Y2	Y2 conditional	Y4	Y5
Food intake	◄►	▲	▲	▼	◄►
Body weight	▲	▼	▼	▼	◄►
Heart rate	◄►	◄►		▼	
Adiposity	▲	▼	▼	▼	▲
Blood pressure	◄►	◄►		▼	
Anxiety		▼			
Nociception	▲				
Bone mass		▲	▲	◄►	
Fertility	▲	◄►		◄►	◄►
Alcohol consumption	▲				
Seizure susceptibility					◄►
Neurogenesis	▼				
References	Howell et al., 2003; Kushi et al., 1998; Naveilhan, 2001; Pedrazzini, 1998	Baldock et al., 2002; Naveilhan et al., 1999; Redrobe et al., 2003; Sainsbury et al., 2002a; Smith-White et al., 2002a,b; Tschenett, 2003	Sainsbury, 2002	Sainsbury, 2002; Smith-White et al., 2002a,b	Marsh et al., 1998, 1999

▲ = increase; ◄► = unaltered; ▼ = decreased.

The generation of transgenic or gene-targeted rodent models offers a valid alternative to these pharmacological approaches. Studies describing the overexpression of neuropeptide Y in transgenic mice and rat models as well as the inactivation of the neuropeptide Y, Y_1, Y_2, Y_4 and Y_5 gene by homologous recombination have been emerged over the recent years. The analysis of the phenotypes of all these animal models has revealed significant and distinct roles of each gene in regulating feeding behavior and energy homeostasis as well as many other functional contributions of Y receptors to different physiological processes. A summary of the current knowledge gained from neuropeptide Y and Y receptor transgenic and knockout models regarding the regulation of energy balance is listed below. Other major phenotypes of these mice models are listed in Tables 1–3.

2. Neuropeptide Y ligand transgenic mouse models

2.1. Neuropeptide Y-overexpressing mice

I.c.v. injections of neuropeptide Y results in a significant increase in food intake and leads to the development of obesity when administered chronically (Sainsbury et al., 1996). Similarly, this can also be seen in the natural mutant ob/ob and db/db mice, both of which have strongly elevated neuropeptide Y levels and are grossly obese. Using the Thy-1 promoter to drive the expression of a mouse neuropeptide Y transgene resulted in the generation of a transgenic mouse model on a BDF1 background with a central nervous system restricted (hippocampus, cerebral cortex, and the arcuate nucleus) modest 115% increase in neuropeptide Y immunreactivity (Inui et al., 1998). Under baseline condition, this relatively little increase in neuropeptide Y did not result in any changes in bodyweight. However, on a sucrose loaded diet, these neuropeptide Y-overexpressing mice exhibit significantly increased body weight gain, transient increased food intake and at approx. 1 year of age develop hyperglycemia and hyperinsulinemia (Kaga et al., 2001), confirming that altered hypothalamic neuropeptide Y expression is important for the development of obesity.

Thiele et al. (1998) also generated a neuropeptide Y-overexpressing mouse model using a construct containing 10 kb of the endogenous mouse neuropeptide Y promoter plus 3 kb of 3′ flanking sequence. This neuropeptide Y transgenic mouse line on FVB background shows approximately five times higher neuropeptide Y mRNA and protein expression in the cortex, amygdala and hippocampus, but interestingly not in the arcuate nucleus of the hypothalamus. These mice have been shown to have a lower level of voluntary alcohol consumption and are more sensitive to the sedative effects of ethanol, opposite what is seen in neuropeptide Y knockout mice (Thiele et al., 2000). No results are available for these mice with regard to food intake and bodyweight regulation.

2.2. Pancreatic polypeptide-overexpressing mice

Pancreatic polypeptide produced in the F cells of the pancreas is released in response to meal eating via vagal initiated mechanism (Hazelwood, 1993). pancreatic polypeptide is also released in response to insulin-induced hypoglycemia and is known to inhibit pancreatic secretion and suppresses gastric and small intestine motility by acting on specific Y_4 receptors in the brain stem thereby controlling vagal output. In this way, pancreatic polypeptide acts to control the entry of nutrients into the circulation by slowing down the digestive progress. Transgenic pancreatic polypeptide overexpressing mice on a mixed BDF1-C57BL/6 background were generated by using the cytomegalovirus immediate early enhancer chicken-beta-actin hybrid promoter to drive a mouse pancreatic polypeptide cDNA followed by the 3′-flanking sequence of the rabbit beta-globin gene (Ueno et al., 1999).

The resulting animals showed strong increases in pancreas specific immunreactivity of pancreatic polypeptide particularly in insulin positive cells. The serum levels of pancreatic polypeptide reached 20 times higher levels in the transgenic animals compared to controls with a much more pronounced phenotype in male compared to female transgenic pancreatic polypeptide mice. Interestingly, these mice exhibited a significant decrease in food intake, both during the dark as well as during the light phase. The rate of gastric emptying was also reduced. Upon i.p. injection of anti-pancreatic polypeptide antiserum in these mice, both effects, the reduction in food intake and gastric emptying, were reversed confirming a role of pancreatic polypeptide as a postprandial satiety signal.

2.3. Neuropeptide Y knockout mice

Erickson et al. (1996a) reported the first knockout of a member of the neuropeptide Y gene family. Replacing the coding sequence of the pre-pro-neuropeptide Y peptide with the lacZ gene and a neomycin-resistant cassette for selection a neuropeptide Y null mouse was generated employing AB1 embryonic stem cells. Functional activity of the LacZ gene in places of where normally neuropeptide Y immunostaining can be found confirmed the successful deletion of the neuropeptide Y gene.

Somewhat unexpected, no gross abnormalities could be detected in these animals. Deletion of the neuropeptide Y gene in these mice did not lead to a reduction in food intake, bodyweight or adiposity under normal condition (Erickson et al., 1996a). Responses to i.c.v. neuropeptide Y injections on food intake were not different in neuropeptide Y deficient mice compared to wild type mice. So were the responses to the anorexic acting corticotropin-releasing-factor (CRF) and melanocortin-4 (MC-4) agonists. However, treatment with leptin, a known negative regulator of hypothalamic neuropeptide Y function, reduced food intake and bodyweight to a greater extend in

the neuropeptide $Y^{-/-}$ mice compared to the controls. However, these mice showed hyperphagic behavior after fasting. This was confirmed by a more recent study by Bannon et al. (2000) who also showed that the neuropeptide Y knockout animals do have a reduced food intake compared to controls in response to fasting supporting an important role of neuropeptide Y in the regulation of energy homeostasis.

A more obvious phenotype can be seen when neuropeptide Y null mice are crossed onto the leptin deficient ob/ob background. These double knockout mice showed a significant reduction of the severe obese phenotype of ob/ob mice (Erickson et al., 1996b) accompanied with reduced food intake, increased energy expenditure and improved serum parameters that influence the development of type II diabetes such as insulin and corticosterone. This confirms that neuropeptide Y is an important central regulator of energy homeostasis that acts downstream of leptin.

Investigations of the effects of neuropeptide Y deficiency on feeding, body weight gain and adiposity was also conducted under conditions of diet or chemical-induced obesity as well as by crossing the neuropeptide Y null mice with mutant obese mice strains other then leptin deficient ob/ob mice (Hollopeter et al., 1998). High fat diet did not seem to have any influence on body weight or food intake in the neuropeptide Y knockout mice compared to the wild type control on a high fat diet. Interestingly, although the neuropeptide Y knockout mice on the high fat diet increased the weight of their fat pads, the actual bodyweight of the mice on the high fat diet was not significantly different from the chow fed controls. Chemical lesions introduced into the arc via mono-sodium-glutamate (MSG) injection into neonates as well as i.p. injections of gold-thio-glucose in adult mice which destroys neurons selectively in the ventral medial hypothalamus (VMH), also known as the satiety center, showed no difference in food intake, bodyweight or fad pad weight between the neuropeptide Y knockout mice and the wild type control mice. Crossing the neuropeptide Y null allele onto a mouse background with decreased energy expenditure due to impaired brown adipose tissue (BAT) function or onto the yellow obese mouse background (A^y), which has a defect in the melanocortin-4 receptor pathway, did not improve the obese phenotype seen in these mice models.

Recently, the effects of neuropeptide Y deficiency has also been investigated in a mouse model which lacks the gene encoding agouti-related peptide (AgRP), an orexigenic peptide acting as an endogenous MC-4 antagonist and which is found to be co-expressed in arcuate neuropeptide Y neurons (Qian et al., 2002). Deletion of the AgRP gene on its own as well as the double mutation does not seem to influence feeding behavior or weight gain under normal condition suggesting that potential compensatory mechanism might have been activated.

3. Y receptor knockout mice

3.1. Y_1 knockout mice

Several laboratories have reported the generation of Y_1 receptor deficient mice (Howell et al., in press; Kushi et al., 1998; Naveilhan et al., 2001; Pedrazzini et al., 1998). Two of the constructs are designed to interrupt the coding sequence with either a 'IRES Tau-LacZ-Neo' (Naveilhan et al., 2001) or a simple neo cassette (Pedrazzini et al., 1998), with the third one actually deleting parts of the gene including the start codon replacing it with a neo cassette (Kushi et al., 1998). The Y_1 receptor targeting construct generated in our laboratory utilizes the cre/loxP technology allowing to produce conditional as well as germline Y_1 knockout mice (Howell et al., in press). Comparison of the different knockout mice is slightly complicated as different targeting strategies and backgrounds of mice have been used. However, all different knockout lines are viable and breed normally.

Y_1 receptor knockout models, similar to neuropeptide Y knockout models, do not show any major abnormalities in regard to food intake. This is slightly surprising as the i.c.v. injection of the Y_1/Y_5 preferring ligand [Leu^{31}/Pro^{34}] neuropeptide Y into normal mice strongly stimulates feeding behavior. However, there are some subtle changes in all Y_1 receptor knockouts analysed. Pedrazzini et al. for example, describes a phenotype of slightly diminished food intake in Y_1 receptor knockout (KO's), both in freely feeding and neuropeptide Y-induced feeding mice. On the other hand, fasting-induced re-feeding is strongly decreased. Interestingly, these mice when adults show an increased content of body fat with no change in protein content. One explanation for that could be the reduced locomotor activity and decreased metabolic rate seen in these mice.

A similar phenotype with no change in basal food intake can be seen in the Y_1 knockout mice generated by Kushi et al. (1998). Again, these mice develop mild hyperinsulinemia and obesity in later life with a significant increase in fat mass particularly in female knockout mice. Thermogenesis, a major component of energy homeostasis, is also altered in these mice. Particularly, the increased levels of uncoupling protein 1 (UCP1) in brown adipose tissue and the reduced levels of UCP2 in white adipose tissue indicate a decrease in energy expenditure.

The germline Y_1 knockout mice generated by us also confirm the previous reported phenotypes including a lack of a significant change in food intake and a late onset obesity syndrome especially in female mice. Brown adipose mass was also increased in both genders indicating a reduction in heat production. Significant increases in white adipose tissue (WAT) could also be observed with females also showing increases in insulin levels but no change in glucose levels. Interestingly, the length of the small intestine in male and female Y_1 knockout mice is significantly reduced. The mRNA expression of neuropeptide Y and

AgRP in the arc of Y_1 knockout mice was not significantly altered, however, the levels of cocaine and amphetamine-regulated transcript (CART) and pro-opiomelanocortin (POMC) mRNA in this area showed a strong decrease compared to wild type controls.

No information regarding the feeding and bodyweight-related phenotype is available from the Y_1 knockout mice generated by Naveilhan et al. (2001). Only results relating to algesia have been reported.

As there is a significant improvement of the obese phenotype in the neuropeptide $Y^{(-/-)} \times$ ob/ob double knockout animals similar crosses with Y receptor knockout mice have been performed to identify the particular Y receptor responsible for this reduction. A $Y_1^{-/-}$,ob/ob double knockout mouse model was generated and described by Pralong et al. (2002). The double knockout mice show a significantly reduced bodyweight in both males and female mice compared to ob/ob mice. However, only the body-weight gain over the first 7 weeks of life has been reported. Interestingly, despite the reduced bodyweight the total fat mass (as a percentage of body weight) in the double knockout mice was still elevated compared to ob/ob mice.

Similarly, $Y_1^{-/-}$,ob/ob double knockout mouse generated in our laboratory also shows a significant reduction in bodyweight, both in females and in males. This is accompanied with significant reduction in the absolute amount of WAT mass in these mice.

3.2. Y_2 knockout mice

Two different Y_2 receptor knockout mice lines have been reported (Naveilhan et al., 1999; Sainsbury et al., 2002a). In the first, the coding sequence of the Y_2 receptor is disrupted by an insertion of a 'IRES Tau-LacZ-Neo' cassette but leaving the translation initiation codon intact. These mice were breed on a mixed 129SV × Balb/c background (Naveilhan et al., 1999). The second Y_2 knockout line generated uses the cre/loxP technology eventually leading to the removal of the entire coding sequence of the Y_2 receptor gene (Sainsbury et al., 2002a). This strain was propagated on a mixed 129SvJ × C57BL/6 background.

Compared to all other Y receptors, the Y_2 receptor is predominately pre-synaptically expressed. It has therefore been proposed that it regulates the synthesis and release of neuropeptide Y and other important neurotransmitters involved in energy homeostasis (King et al., 2000). Furthermore, expression of the Y_2 receptor in an area of the arcuate nucleus with a permeable blood brain barrier, accessible to circulating factors, makes it an ideal candidate to mediate peripheral neuropeptide Y/peptide YY signals on the regulation of energy homeostasis. The phenotypes described for the two Y_2 knockout mouse lines differ. Naveilhan et al. describes a phenotype of increased body weight, food intake and fat deposition accompanied with an attenuated response to leptin in female mice. Neuropeptide Y-induced food intake and the re-feeding responses after 24-h starvation were normal. In contrast, the germline Y_2 knockout model described by Sainsbury et al. (2002a) shows a reduced bodyweight gain and adiposity in male and female mice. Food intake was unaltered in male and increased in female knockout mice with re-feeding after starvation strongly elevated in both genders. Hypothalamic neuropeptide Y and AgRP mRNA levels were significantly elevated, whereas POMC and CART mRNA in the arc and CRH in the PVN were significantly decreased. Serum levels of pancreatic polypeptide where increased by two- to three-fold possibly contributing to the leaner phenotype similarly to what can be seen in the PP overexpressing mice. In addition, corticosterone levels were reduced.

I.p. injection of the endogenously produced and post-prandially released Y_2/Y_5 preferring agonist peptide YY-(3–36), which inhibits food intake in wild type mice, fails to do so in Y_2 knockout mice (Batterham et al., 2002). This strongly suggests a gut-hypothalamic pathway that regulates food intake via arcuate Y_2 receptors. This was also confirmed by hypothalamus-specific deletion of Y_2 receptors in conditional Y_2 knockout mice. A significant decrease in body weight and a significant increase in food intake that is associated with increased mRNA levels for the orexigenic neuropeptide Y and AgRP, as well as the anorexic POMC and CART in the arcuate nucleus, can be seen in these mice (Sainsbury et al., 2002a). The changes in peptide expression persist over a period of 4 weeks after Y_2 deletion, however, the effect on body weight and food intake subsided within this time. The transience of the observed effects on food intake and body weight in the hypothalamus-specific Y_2 knockout mice compared to germline Y_2 knockout mice underlines the importance of conditional models of gene deletion, as developmental, secondary, or extra-hypothalamic mechanisms may mask such effects in germline knockouts.

Crossing the $Y_2^{-/-}$ mice onto the ob/ob background has no effect on food intake and body weight, however, it attenuates the increased adiposity, hyperinsulinemia, hyperglycemia, and increased hypothalamo-pituitary-adrenal (HPA) axis activity seen in ob/ob mice (Sainsbury et al., 2002b). Hypothalamic POMC mRNA expression is increased in $Y_2^{-/-}$,ob/ob double knockout mice but has no effect on neuropeptide Y, AgRP, or CART expression. Alterations in melanocortin tonus in the arcuate nucleus, and/or effects on the hypothalamo-pituitary-adrenal axis therefore might be the reason for the improvement of the type-2 diabetes phenotype of ob/ob mice after Y_2 deletion. Interestingly, the reduction in WAT mass in $Y_2^{-/-}$,ob/ob double knockout mice is compensated for by increased lean mass.

Y_2/Y_4 double knockout mice have also been generated (Sainsbury et al., 2003). Surprisingly, despite significant hyperphagia [$Y_2^{-/-}$, $Y_4^{-/-}$], mice retained a markedly lean phenotype, with reduced body weight, white adipose tissue mass, leptinemia, and insulinemia. These changes were more pronounced than those observed in $Y_2^{-/-}$ mice

suggesting synergy between Y_2 and Y_4 receptor pathways. The double knockout thus reveals a level of complexity in the regulation of Y receptor-mediated signals not made apparent in previous single knockout models. A lack of feedback inhibition from post-prandially released factors such as peptide YY-(3–36) and pancreatic polypeptide, each of which have been shown to reduce food intake in humans and rodents by acting on central Y_2 and possibly Y_4 receptors, may be responsible for the strong increase in food intake in these mice (Batterham et al., 2002). The reduction in BAT mass, indicative of enhanced thermogenesis and increased metabolic rate, is most likely the reason for the lean phenotype seen.

3.3. Y_4 receptor knockout mice

Little efforts have been devoted to investigate the physiological function of the Y_4 receptor making it the least understood member of the Y receptor family. The Y_4 receptor is found mostly in peripheral tissues, however, there are also significant levels of expression of Y_4 receptor found in areas known to be important in the regulation of food intake and energy homeostasis such as the PVN and some brain stem nuclei (Parker and Herzog, 1999). The ligand with the highest affinity for the Y_4 receptor is pancreatic polypeptide, however, neuropeptide Y and peptide YY can also activate the receptor when reaching high enough concentrations. So far, only one report which describes the generation and characterisation of conditional and germline Y_4 knockout mouse strains on a mixed 129SvJ/C57BL/6 background is available (Sainsbury et al., 2002c).

Germline male and female Y_4 knockout mice show a slightly but significant reduced bodyweight gain accompanied with reduced food intake (Sainsbury et al., 2002c). Male Y_4 receptor knockout mice have also reduced white adipose tissue mass as well as strongly elevated plasma levels for pancreatic polypeptide, the proposed endogenous high affinity ligand for this Y receptor. Other serum parameters important in the regulation of energy homeostasis such as insulin, glucose and corticosterone were not significantly altered in the Y_4 receptor knockout mice. Furthermore, no changes in the level of expression of mRNAs for neuropeptide Y, AgRP, CART and POMC in the arcuate nucleus could be detected.

Interestingly, despite the fact that the Y_4 receptor knockout mice show a lean phenotype, Y_4 deficiency has no beneficial effects to reduce body weight or excessive adiposity of ob/ob mice neither does it reduce food intake in these mice (Sainsbury et al., 2002c). In the contrary, male $Y_4^{-/-}$,ob/ob double knockout mice actually seem to have a tendency to even higher bodyweight compared to ob/ob mice. However, the WAT mass in the $Y_4^{-/-}$, ob/ob double knockout mice is not different to ob/ob mice so the additional weight is probably contributed by lean mass, which is consistent with the elevated testosterone levels in these mice.

3.4. Y_5 receptor knockout

The Y_5 receptor, once praised as the ‘feeding receptor’, has also been deleted in a knockout mouse model. Marsh et al. (1998) generated these mice on a 129Sv/C57BL/6 background in which the coding sequence of the Y_5 receptor was replaced with a Tau-LacZ cassette for visualisation of gene expression. Germline deletion of the Y_5 receptor does not provide any evidence for a major role of this Y receptor in feeding (Marsh et al., 1998). The Y_5 knockout mice feed and grow normally when young and actually develop late onset (>30 weeks) obesity with an increase in food intake and bodyweight. Body temperature was also not affected suggesting a normal metabolic rate. Also, fasting-induced re-feeding pattern is not different in the Y_5 knockout mice compared to wild type mice. However, responses to i.c.v. neuropeptide Y or neuropeptide Y analogs are either reduced or missing, which is more obvious when a higher dose of neuropeptide Y is employed. Interestingly, administration of neuropeptide Y and the Y_1 antagonist 1229U91 together completely abolishes any effect of neuropeptide Y on feeding suggesting that a combined action of both Y_1 and Y_5 receptors is necessary to elicit the strong stimulatory effect of neuropeptide Y on food intake.

Crossing of the Y_5 knockout mice with ob/ob mice did not improve the obese phenotype of the ob/ob mice (Marsh et al., 1999). In both genders, food intake, bodyweight and adiposity were not different from ob/ob mice suggesting that the Y_5 receptor is not required for neuropeptide Y’s action to induce obesity in leptin deficient ob/ob mice.

3.5. Summary on Y receptor involvement in energy homeostasis

Considering the massive increase in food intake and body weight gain seen in rodent models after i.c.v. infusion of neuropeptide Y, the finding that most of the germline knockout models for members of the neuropeptide Y family have little or no obvious feeding-related phenotype came as somewhat of a surprise. However, it is possible that removing of one component of the neuropeptide Y family system leads to adaptive changes during development in order to maintain homeostasis within the system. Modification in these mutant mice may be subtle and needs to be investigated further. Y receptors show overlapping expression in important areas of the regulation of energy homeostasis and they also have similar affinity for neuropeptide Y. This redundancy in the system could lead to compensatory responses when one of the components is missing. Local expression levels of the remaining Y receptors in a particular Y receptor knockout model need to be checked to better define the role of the missing specific Y receptor.

For example, the expression and binding of neuropeptide Y receptors in neuropeptide Y knockout mice was investigated by in situ hybridization and receptor auto-radiography using $^{(125)}$I-[Leu^{31},Pro^{34}]peptide YY and $^{(125)}$I-peptide YY-

Table 3
Double Y receptor knockout models

	Y1/Y2	Y2/Y4	Y1/ob	Y2/ob	Y4/ob	Y5/ob
Food intake		▼	◄►	◄►	▲	◄►
Body weight		▼	▼	◄►	▲	◄►
Adiposity		▼	▼	◄►	▼	◄►
Nociception	▲					
Bone mass		▲				
Fertility			▲	◄►	▲	◄►
References	Naveilhan et al., 2001	Sainsbury et al., 2003	Pralong et al., 2002	Sainsbury et al., 2002a,b,c; Naveilhan et al., 2002	Sainsbury et al., 2002a,b,c	Marsh et al., 1998

▲ = increase; ◄► = unaltered; ▼ = decreased.

(3–36) as radioligands (Trivedi et al., 2001). In the hippocampus, no significant changes could be detected for Y_1, Y_4, Y_5 and y_6 receptors, however, a six-fold increase in Y_2 receptor mRNA was observed in the CA1 region. In addition, an increase of between 60% and 400% of Y_2 receptor binding in several brain areas was detected. In the hypothalamus, Y_1 receptor binding was also increased giving further evidence that the lack of the ligand gene can lead to alterations in Y receptor levels (Trivedi et al., 2001).

Radio-ligand binding assays with Y receptor preferring ligands also detected alterations in the expression levels of other Y receptors in the Y_2 knockout model. Particularly the binding of the Y_2/Y_5 preferring ligand ^{125}I-peptide YY-(3–36) was only slightly above background suggesting the absence or strongly diminished function of Y_5 receptor in these mice (Sainsbury et al., 2002b).

One must also consider that the neuropeptide Y system is closely interacting with several other neuropeptides such as AgRP, POMC and CART to regulate energy homeostasis. Deletion of neuropeptide Y or a particular Y receptor can trigger that such other pathways take over some of the regulatory functions by increasing or decreasing the expression of a particular neurotransmitter. Such changes of mRNA expression levels of neuropeptide Y, AgRP, POMC, CART, TRH and CRH as have been seen in Y_2 and Y_2/Y_4 double knockout mice can help explain some of the observed phenotypical changes (Sainsbury et al., 2002a,b,c).

In summary, results from all these transgenic models do indicate that the Y_1 receptor is still the most likely candidate to mediate the strong stimulatory activity of neuropeptide Y on food intake, whereas the Y_2 and Y_4 either have an inhibitory or no effect on this parameter. However, these two Y receptors do have a strong influence on the regulation of energy homeostasis due to the feedback action of their gut-derived ligands. Y_5 receptors on the other hand only contribute slightly to the stimulation of food intake and do not have any significant importance under conditions when leptin signaling is impaired. It is also important to keep in mind that in the mouse genome, a further functional Y receptor, y_6 exists and it remains to be seen what role the y_6 receptor plays in the regulation of energy homeostasis. Investigations of additional double knockout models including Y_1/Y_5 double mutant mice may also help to shed more light onto this question.

4. Other neuropeptide Y and Y receptor knockout phenotypes

The diverse function in the central and peripheral nervous system in which the neuropeptide Y system is involved additionally to the regulation of energy homeostasis makes it very clear that other phenotypical changes should be expected in the different transgenic models. A large number of articles reporting the identification of such additional functional contributions from the neuropeptide Y system to a variety of physiological processes have emerged (reviewed by Berglund et al., 2003; Thorsell and Heilig, 2002). However, the detailed description of all of these additional findings would exceed the space and would also be somewhat outside the scope of this review on energy homeostasis, therefore, only a summary on this findings is given in Tables 1–3.

5. Concluding remarks

Studies on transgenic models of neuropeptide Y and its Y receptors have provided a wealth of information about molecular and behavioral phenotypes over recent years finally defining more clearly some of the roles for the individual Y receptors. With the help of these knockout and transgenic models, important new insights have been revealed particularly in the coordinated regulation of food intake and energy homeostasis. Some of the findings in the transgenic models, however, did not show the expected ‘severe’ phenotypes that would have been predicted from previously conducted pharmacological intervention studies. The relative ‘normal’ feeding phenotype of the neuropeptide $Y^{-/-}$ mouse in particular was a surprise considering the massive increase in food intake and bodyweight gain

observed after acute and chronic neuropeptide Y administration. The discrepancies between pharmacological and transgenic research outcomes could have several reasons. Redundancy in the system, which could lead to compensatory changes in knockout animals during development, might be one of it. One other factor sometimes overlooked is that neuropeptide Y injected i.c.v. can reach a large number of different sites in the brain, not all of which are involved in the regulation of energy homeostasis. Stimulation of all kinds of Y receptors in many different locations at once might not represent a 'normal' physiological response and might as such not show the true functional consequence of the activation of a particular Y receptor pathway. Similarly, germline deletion of a gene causes the loss of function of that gene in all tissues where it is normally expressed producing a phenotype that is compiled by the sum of all lost functions. In addition, deletion of a gene in the germline can also produce secondary effects not directly linked to the actual function of the deleted gene.

Some refinement of the in vivo targeting mutagenesis techniques will be needed to circumvent these problems. The use of knock-in strategies, which will allow to modify rather then to completely inactivate receptor or ligand functions, will be required. The generation of a full set of conditional knockout models for all Y receptors and ligands should also help to overcome the inherent problem of developmental influences on the phenotypes. More importantly, this will allow to selectively delete the genes in only a defined tissue or nucleus in an adult animal thereby avoiding complication due to whole body ablation.

Some transgenic models for members of the neuropeptide Y gene and Y receptor family have still to be generated including knockout models for peptide YY and pancreatic polypeptide as well as y_6. Even as the y_6 receptor is considered to be a pseudo-gene in primates in the mouse system, it is a functional entity and needs to be analysed in the same way as all other Y receptor knockout models. Nevertheless, the data accumulated from these neuropeptide Y transgenic models are already of great value and will provide the basis for future, more detailed studies on the complex nature of neuropeptide Y physiology. Furthermore, knockout models are an invaluable source for the testing of the specificity of agonists and antagonist or to determine the specificity of antibodies.

Finally, with the fast progress on the development of gene array technologies, proteomic approaches and other bioinformatic tools, these neuropeptide Y and Y receptor knockout and transgenic mouse models will be extremely useful for the identification of molecular networks that contribute to certain complex physiological processes. Studies employing such technologies, when carefully conducted, may identify a great number of new candidate genes involved in many different neuropeptide Y-mediated functions not yet known or associated with these systems.

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