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Review

Both overexpression of agouti-related peptide or neuropeptide Y in the paraventricular nucleus or lateral hypothalamus induce obesity in a neuropeptide- and nucleus specific manner

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ABSTRACT

Both reduction of melanocortin signaling and increase in neuropeptide Y signaling in the brain result in obesity. However, where in the brain reduced melanocortin or increased neuropeptide Y signaling mediate these effects is poorly understood. In separate experiments we have injected recombinant adeno-associated viral vectors that overexpressed agouti-related peptide or neuropeptide Y in specific brain regions namely the paraventricular nucleus and the lateral hypothalamus. In this review we compare the results from these studies and discuss these data with previous data from intracerebroventricular or local brain injections. This review shows that the effects of agouti-related peptide clearly differ from those of neuropeptide Y. In addition, these data suggests complementary roles for these neuropeptides in energy balance.

Contents

1. Introduction

The brain integrates a wide range of informative stimuli for nutritional state and for energy levels of the body, to produce appropriate responses in terms of food intake and energy expenditure to maintain a stable body weight (homeostatic control) (Cone, 2005; Ellacott and Cone, 2006). In Western societies, there is plenty of palatable food available and there is little need to exercise. Together this drives the energy balance towards a positive one, with obesity as a result.

Already in the 1950's, brain lesions and stimulation studies identified the hypothalamus as a major brain area controlling energy homeostasis (Anand and Brobeck, 1951; Stellar, 1954). However, it

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has become evident that the hypothalamic regulation of energy balance involves a complex network of integrated pathways. The arcuate nucleus in the hypothalamus is an important nucleus for integration of signals related to energy status, such as leptin and insulin, and is involved in the initiation of a feeding response via projections to hypothalamic and extra-hypothalamic areas such as the hindbrain (reviewed in (Schwartz et al., 2000; Grill, 2010)). The arcuate nucleus is highly sensitive to peripheral signals, such as adiposity signals leptin and insulin, because it is anatomically located in close proximity to the blood stream (Norsted et al., 2008). In addition, when the arcuate nucleus is destroyed leptin can no longer reduce food intake (Tang-Christensen et al., 1999; Dawson et al., 1997). In the arcuate nucleus there are two important groups of neurons; one population synthesizes the anorexigenic neuropeptides pro-opiomelanocortin and cocaine- and amphetamine-regulated transcript (Vrang et al., 1999), while the other population produces the orexigenic neuropeptides agouti-related peptide and neuropeptide Y (Hahn et al., 1998). Pro-opiomelanocortin is the precursor of endogenous melanocortins, agonists for melanocortin receptors. Interestingly, the agouti-related peptide acts as an inverse agonist at melanocortin receptors. Thus, the activity of melanocortin receptors is tightly controlled by melanocortins and agouti-related peptide, that both originate from neurons that have their cell bodies in the arcuate nucleus. Pro-opiomelanocortin/cocaine- and amphetamine-regulated transcript neurons and agouti-related peptide/neuropeptide Y neurons react in opposite manners to different signals, such as leptin or insulin (reviewed in (Belgardt et al., 2009)). Although agouti-related peptide and neuropeptide Y are both orexigenic they probably use different mechanisms to affect energy balance. *In vivo* drug administration studies and knockout models in rodents have greatly contributed to the knowledge concerning the role of neuropeptide Y and melanocortin signaling in the modulation of energy homeostasis.

Most pharmacological and knockout studies have focused on the effects of neuropeptides or their receptors in whole brain by infusing the peptides intracerebroventricular (i.c.v.) or investigating whole body knockout mice. However, when a drug is administrated i.c.v., the drug can bind to its receptors in different brain regions, which contrasts with physiological release of neuropeptides at projection areas of depolarizing neurons. In case of transgenic overexpression or knockout of a gene, the gene is expressed ubiquitous including areas where it normally is not expressed, or the gene is deleted all over the body. Preferably one would like to locally interfere with neuropeptide signaling in a cell type and/or nucleus specific manner. Local infusion studies with permanent cannulae in the brain have been done, but the drugs can only be infused for up to a week with minipumps connected to cannulae and the ligand may diffuse further into the brain then intended. Long-term studies are complicated because over longer periods these cannulae get blocked. Conditional expression or deletion of neuropeptide genes and receptors have clearly contributed further to our understanding of which cell types are implicated in melanocortin and agouti-related peptide mediated effects. However, conditional expression depends on the availability of cell type and nucleus specific promoters that are able to drive Cre-expression and it is thus far only possible in mice. In this review we discuss the results which were obtained via viral vector mediated gene transfer as a complementary approach to study the function of agouti-related peptide and neuropeptide Y in regulation of body weight, food intake, meal patterns, locomotor activity, core temperature, endocrine parameters and neuropeptide expression in specific brain areas. In addition, these data are compared to results obtained via knockout and i.c.v. administration studies.

1.1. Agouti-related peptide

Agouti-related peptide is a 132 amino acid long peptide, which is predominantly expressed in the arcuate nucleus (Shutter et al., 1997). This pre–pro-neuropeptide is processed into different parts by prohormone convertase mediated splicing (Creemers et al., 2006). The C-terminal part of agouti-related peptide, agouti-related peptide$_{83-132}$, can bind to the melanocortin MC_3 receptor or melanocortin MC_4 receptor and increases food intake (Creemers et al., 2006; Quillan et al., 1998; Goto et al., 2003). The N-terminal parts cannot bind the melanocortin MC_3 receptor or melanocortin MC_4 receptor, but they were reported to decrease energy expenditure (Goto et al., 2003). The expression of agouti-related peptide in the arcuate nucleus is decreased by positive energy balance and increased leptin levels (Mizuno et al., 1999; Mizuno and Mobbs, 1999; Korner et al., 2001). In contrast, agouti-related peptide expression is increased by negative energy balance and reduced leptin levels. Agouti-related peptide is upregulated in leptin deficient (ob/ob) mice and other models for obesity or diabetes (Ollmann et al., 1997; Shutter et al., 1997). Agouti-related peptide neurons project to many areas in the brain (Broberger et al., 1998; Blouet and Schwartz, 2010). In these areas agouti-related peptide is released and binds to melanocortin MC_3 receptor and/or melanocortin MC_4 receptor (Roselli-Rehfuss et al., 1993; Gantz et al., 1993; Mountjoy et al., 1994; Kishi et al., 2003). It exerts its functions by acting as an inverse agonist on the constitutive active melanocortin receptors and antagonizing the endogenous melanocortins (α-melanocyte stimulating hormone (α-MSH), β-MSH, γ-MSH and adrenocorticotropic hormone), for binding to these receptors (Ollmann et al., 1997; Nijenhuis et al., 2001; Haskell-Luevano and Monck, 2001). In this way a delicate balance is established to regulate energy homeostasis via energy intake and energy expenditure (Roselli-Rehfuss et al., 1993; Poggioli et al., 1986; Kask et al., 2000; Abbott et al., 2000; McKay et al., 1981; Fan et al., 1997; Giraudo et al., 1998; Rossi et al., 1998; Ollmann et al., 1997; Jonsson et al., 2002; Small et al., 2001; Brito et al., 2007; Murphy et al., 2000; Chen et al., 2000a,b).

Transgenic overexpression of the agouti-related peptide gene or i.c.v. administration of agouti-related peptide $_{83-132}$ increases food intake and body weight of mice significantly (Ollmann et al., 1997; Graham et al., 1997; Rossi et al., 1998; Kim et al., 2002; Small et al., 2001), while agouti-related peptide knockout mice show relatively normal ingestive behavior and body weight (Qian et al., 2002). In contrast, deletion of the melanocortin MC_3 receptor or melanocortin MC_4 receptor had clear effects on energy homeostasis. Deletion of melanocortin MC_3 receptor modestly affects feeding behavior, but these animals are more efficient in storing energy, i.e. they need fewer calories to gain a gram of body weight and they have increased adipose mass while body weight is only modestly affected (Chen et al., 2000a,b; Butler et al., 2000). Melanocortin MC_4 receptor knockout mice show hyperphagia, decreased energy expenditure and are obese (Huszar et al., 1997; Ste et al., 2000; Chen et al., 2000a,b). Melanocortin MC_4 receptor$^{-/-}$ mice are not sensitive for intake suppressive effects of Melanotan-II (MT-II, a melanocortin agonist), underscoring the role of melanocortin MC_4 receptor in food intake (Chen et al., 2000a,b; Marsh et al., 1999a,b). Nevertheless, other receptors also are implicated in regulation of food intake since administration of agouti-related peptide to melanocortin MC_4 receptor $^{-/-}$ mice still induces a small increase in food intake (Fekete et al., 2004; Marsh et al., 1999a,b). In addition, the melanocortin MC_4 receptor knockout mice are obese before the onset of hyperphagia, suggesting that a decrease in energy expenditure contributes to the melanocortin MC_4 receptor $^{-/-}$ phenotype (Ste et al., 2000). The food intake and energy expenditure effects of the melanocortin MC_4 receptor may be located in different brain areas; since re-expression of MC_4 receptor in the paraventricular hypothalamus and amygdala corrected the hyperphagia, but only partially reduced obesity (Balthasar et al., 2005). To more specifically determine in which brain areas agouti-related peptide increases food intake or decreases locomotor activity we recently overexpressed agouti-related peptide in different hypothalamic areas with an adeno-associated viral vector (de Backer et al., in press). Thus, we overexpressed agouti-related

peptide in order to locally reduce melanocortin signaling in a wild type rat, as a complementary approach to the re-expression of melanocortin MC_4 receptor in a melanocortin MC_4 receptor knockout background in mice (Balthasar et al., 2005).

1.2. Neuropeptide Y

Neuropeptide Y is a 36 amino acid long peptide and is expressed widely in the brain. High numbers of neuropeptide Y neurons are located in the arcuate nucleus (Allen et al., 1983). Dorsomedial hypothalamic neurons also produce neuropeptide Y, but these neurons differ from the neuropeptide Y neurons in the arcuate nucleus (Draper et al., 2010). In the arcuate nucleus neuropeptide Y is co-localized with agouti-related peptide (Hahn et al., 1998). Neuropeptide Y is implicated in several physiological processes including anxiety, reproduction and energy homeostasis.

Neuropeptide Y binds to Y_1, Y_2, Y_4 and Y_5 receptor in humans and rodents, but the contribution of the different receptor subtypes to regulation of energy balance is difficult to assess due to the lack of highly selective agonists and antagonists. All subtypes are found in hypothalamic areas (Parker and Herzog, 1999; Fetissov et al., 2004; Larsen and Kristensen, 2000; Gerald et al., 1996; Larsen and Kristensen, 1998). However, the feeding effects of neuropeptide Y are most likely mediated through signaling via Y_1 and Y_5 receptors. i.c.v. administration of neuropeptide Y increases food intake and reduces energy expenditure (Clark et al., 1984; Morley et al., 1987; Bouali et al., 1995; Jolicoeur et al., 1995; Baran et al., 2002; Zarjevski et al., 1993; Heilig and Murison, 1987). However, overexpression or removal (as in knockouts) of neuropeptide Y does not affect food intake or body weight on regular chow obviously (Inui et al., 1998; Ste et al., 2005; Erickson et al., 1996a,b, 1997). When analyzed more carefully neuropeptide Y deficient mice do have an attenuated feeding response to fasting and leptin administration (Erickson et al., 1996a,b; Bannon et al., 2000; Hollopeter et al., 1998; Sindelar et al., 2005). In addition, when neuropeptide Y transgenic mice were placed on a high sucrose diet these mice displayed hyperphagia and increased body weight (Kaga et al., 2001). In contrast to the expectations, genetic removal of Y_1 or Y_5 receptors increased body weight (Pedrazzini et al., 1998; Kushi et al., 1998; Kanatani et al., 2000; Marsh et al., 1998; Raposinho et al., 2004). Removal of Y_4 receptors resulted in a lean phenotype, although this receptor is not thought to play a major role in the regulation of energy homeostasis (Sainsbury et al., 2002b). The effects of deletion of Y_2 receptor are contradictory (Naveilhan et al., 1999; Sainsbury et al., 2002a). It has been suggested that neuropeptide Y receptor subtypes compensate one and another after deletion of one subtype (Lin et al., 2006), which complicates interpretation of results from knockout mice. To investigate the local effect of neuropeptide Y in the paraventricular hypothalamus and lateral hypothalamus our group has overexpressed neuropeptide Y in these target areas and investigated the effects on energy homeostasis (Tiesjema et al., 2007). Three weeks (around day 21) and six weeks (around day 40) after virus injection, food patterns, locomotor activity and body temperature were monitored.

1.3. Effects of agouti-related peptide and neuropeptide Y on body weight and fat mass (Table 1)

1.3.1. Paraventricular hypothalamus

Rats with adeno-associated viral vector-mediated neuropeptide Y overexpression in the paraventricular hypothalamus (AAV-PVN-NPY rats) accumulated 67% more body weight than controls (AAV-empty) 50 days post injection. In addition, AAV-PVN-NPY rats gained 3 times more subcutaneous white adipose tissue (SWAT) and 2 times more abdominal white adipose tissue (AWAT). Thus, they showed an obese phenotype. Adeno-associated viral vector-mediated overexpression of agouti-related peptide in the paraventricular hypothalamus (AAV-PVN-AgRP) increased body weight of rats with 80% compared to controls (AAV-GFP) at day 50. In addition, at the end of the experiment the SWAT and AWAT was increased 2-fold compared to the controls.

1.3.2. Lateral hypothalamus

In the lateral hypothalamus overexpression of neuropeptide Y (AAV-LH-NPY) increased body weight with 127% compared to controls at day 50 post injection. These rats accumulated more body weight than AAV-PVN-NPY rats but their increase in SWAT and AWAT was similar (3-fold and 2-fold, respectively). Rats with adeno-associated viral vector mediated overexpression of agouti-related peptide in the lateral hypothalamus (AAV-LH-AgRP) accumulated 80% more body weight than their controls and their SWAT and AWAT was increased 2 times compared to the control group.

These data are in agreement with i.c.v. infusions studies of neuropeptide Y or agouti-related peptide where infusion of these peptides for 1 week resulted in obesity and increased fat mass (Baran et al., 2002; Raposinho et al., 2000). In addition, chronic site specific administration of neuropeptide Y, for 5–7 days, in the paraventricular hypothalamus or lateral hypothalamus increased body weight (McMinn et al., 1998; Raposinho et al., 2001). However, these chronic administration studies were not as long-term as our studies thus we cannot compare these studies one to one. One study investigated long-term effects of injections with adeno-associated viral vector expressing antisense neuropeptide Y cRNA into the arcuate nucleus of adult rat males. This study showed that suppression of neuropeptide Y by 50–60% reduced body weight and food intake until the end of the experiment (50 days p.i.) (Gardiner et al., 2005). Together, these data show that agouti-related peptide and neuropeptide Y both increase body weight and fat mass.

1.4. Effects of agouti-related peptide and neuropeptide Y on food intake and meal patterns (Tables 1 and 2)

1.4.1. Paraventricular hypothalamus

Adeno-associated viral vector mediated overexpression of neuropeptide Y in the paraventricular hypothalamus only temporarily increased food intake. After approximately 20 days food intake reduced and food intake was returned to control levels on day 40. A subsequent pair-fed study by our group showed that the food intake was increased in AAV-PVN-NPY rats until a certain body weight was achieved (Tiesjema et al., 2009). The transient increase in food intake was due to an increase in meal frequency in the light phase.

Table 1
Comparison of parameters affected by AAV-NPY or AAV-AgRP overexpression in different brain areas at 40/48 days p.i.

DAY 40/48	PVN-AgRP	PVN-NPY	LH-AgRP	LH-NPY
Body weight gain[a]	↑ 80%	↑ 67%	↑ 80%	↑ 127%
Fat %	↑	↑	↑	↑
Food intake	↑	L↑	↑	L↑
Meal size	↑	–	↑	↑
Meal frequency	L↑	–	–	–
Water intake	↑	–	–	–
Body temperature	D↓	D↓	–	D↓
Locomotor activity	D↓[b]	D↓	–	D↓
Plasma leptin	↑ (~38 ng/ml)	↑	↑ (~19 ng/ml)	↑
Plasma insulin	↑	↑	↑	↑
Plasma glucose	–	↑	–	–

↑ increased by AgRP or NPY overexpression; ↓ decreased by AgRP or NPY overexpression.

[a] % increase in body weight compared to controls at end of the experiment. L: light phase; D: dark phase.

[b] Data to be interpreted with care. Data from references: Tiesjema et al. (2007) and de Backer et al. (in press).

Table 2
Comparison of parameters affected by AAV-NPY or AAV-AgRP overexpression in different brain areas at 20/21 days p.i.

DAY 20/21	PVN-AgRP	PVN-NPY	LH-AgRP	LH-NPY
Body weight gain	↑	↑	↑	↑
Food intake	↑	L↑	↑	L↑
Meal size	↑	–	↑	↑
Meal frequency	L↑	L↑	–	–
Water intake	↑	↑	–	–
Body temperature	–	L↑, D↓	–	L↑, D↓
Locomotor activity	D↓[a]	D↓	–	↓

↑ increased by AgRP or NPY overexpression; ↓ decreased by AgRP or NPY overexpression; L: light phase; D: dark phase.
[a] Data to be interpreted with care. Data from references: Tiesjema et al. (2007) and de Backer et al. (in press).

In contrast, overexpression of agouti-related peptide in the paraventricular hypothalamus increased daily food intake of rats during the entire experiment. The increase in food intake was due to increased average meal size, while average meal frequency was normal. Careful examination of the meal patterns in light and dark period showed that, in addition to increased meal size in light and dark period, AAV-PVN-AgRP rats had a slight, but significant, increase in meal frequency in the light period compared to controls.

1.4.2. Lateral hypothalamus

In the lateral hypothalamus both overexpression of agouti-related peptide or neuropeptide Y increased food intake during the entire experiment. AAV-LH-NPY rats increased their food intake due to an increase in meal size in light and dark phase. In addition, at day 48 meal frequency was increased in light phase and decreased in dark phase, thus showing a disturbance of normal circadian rhythm in food intake. AAV-LH-AgRP rats showed an increase in meal size in dark and light phase and no alterations in meal frequency.

The effects of agouti-related peptide overexpression are in agreement with infusion studies which showed that antagonists and agonist for melanocortin MC_3 receptor and/or melanocortin MC_4 receptors affect meal size and not frequency (Tang-Christensen et al., 2004; Santollo and Eckel, 2008; Zheng et al., 2005; Azzara et al., 2002; Williams et al., 2002; Adan et al., 2006). In addition, humans with melanocortin MC_4 receptor deficiency eat larger meals during an *ad libitum* test meal this indicates a decrease in satiation (Farooqi et al., 2003). Infusion of neuropeptide Y increases both meal frequency and meal size (Day et al., 2005; Marin Bivens et al., 1998; Leibowitz and Alexander, 1991).

These data suggest that overexpression of agouti-related peptide in the paraventricular hypothalamus and lateral hypothalamus decreases satiation, as shown by the predominant increase in meal size. However, the effects of neuropeptide Y overexpression appear to be different between the paraventricular hypothalamus and lateral hypothalamus. In the paraventricular hypothalamus neuropeptide Y regulates initiation of food intake in light phase, while in the lateral hypothalamus neuropeptide Y regulates satiation in both light and dark phase.

1.5. Effects of agouti-related peptide and neuropeptide Y on locomotor activity and body temperature (Tables 1 and 2)

1.5.1. Paraventricular nucleus

Neuropeptide Y overexpression in the paraventricular hypothalamus reduces locomotor activity in the dark phase of day 21 and 48 compared to controls. AAV-PVN-NPY decreased average core temperature in light and dark phase of day 21 and in the dark phase of day 48. In addition, diurnal rhythmicity in core temperature in AAV-PVN-NPY rats was reduced. The effects of adeno-associated viral vector mediated overexpression of agouti-related peptide on locomotor activity are not as clear as the effects of neuropeptide Y overexpression in the hypothalamic areas. On day 21 and 40 post injections, agouti-related peptide overexpression in the paraventricular hypothalamus appears to reduce locomotor activity in the dark phase, however in this particular experiment, the control groups show high locomotor activity levels thus this data has to be interpreted with care. AAV-PVN-AgRP rats showed a decreased core temperature in the dark phase on day 40 post injection.

1.5.2. Lateral hypothalamus

In the lateral hypothalamus neuropeptide Y overexpression also reduced locomotor activity in the dark phase of day 21 and 48, but there was also a reduction in locomotor activity in the light phase of day 21. In addition, neuropeptide Y overexpression in the lateral hypothalamus also decreased core temperature in the light and dark phase of day 21 and in the dark phase of day 48. Agouti-related peptide overexpression in the lateral hypothalamus, however, does not significantly alter locomotor activity or core temperature.

Chronic neuropeptide Y administration to the paraventricular hypothalamus does appear to result in hypothermia (Stanley et al., 1986). However, acute neuropeptide Y infusions in the paraventricular hypothalamus showed that neuropeptide Y increased body temperature while low doses of neuropeptide Y infused in the lateral hypothalamus resulted in hyperthermia while high doses resulted in hypothermia (Bouali et al., 1995; Jolicoeur et al., 1995). Locomotor activity is decreased by acute i.c.v. administration of neuropeptide Y (Heilig and Murison, 1987; Heilig et al., 1989).

Previous studies reported a decrease in locomotor activity and core body temperature after i.c.v. administration of agouti-related $peptide_{83–132}$ (Tang-Christensen et al., 2004; Creemers et al., 2006) and 6 month old mice agouti-related peptide knockout mice were reported to be hyperactive (Wortley et al., 2005). However, re-expression of melanocortin MC_4 receptor in the paraventricular hypothalamus and amygdala of melanocortin MC_4 receptor knockout mice had no effects on locomotor activity (Balthasar et al., 2005). Melanocortin MC_3 receptor $^{-/-}$ mice did show a decrease in locomotor activity and melanocortin MC_3 receptors are also expressed in the paraventricular hypothalamus (Chen et al., 2000a,b; Roselli-Rehfuss et al., 1993; Butler et al., 2000). Therefore, reductions in locomotor activity by agouti-related peptide overexpression in the paraventricular hypothalamus may be due to inhibition of melanocortin MC_3 receptors. However, one cannot exclude that re-expression of melanocortin MC_4 receptor in a knockout background (Balthasar et al., 2005) masks effects of melanocortins and agouti-related peptide in the paraventricular hypothalamus on locomotor activity.

Thus, neuropeptide Y in the paraventricular hypothalamus and lateral hypothalamus appears to reduce locomotor activity and core temperature, while agouti-related peptide in the paraventricular hypothalamus may reduce locomotor activity and temperature.

1.6. Effects of agouti-related peptide and neuropeptide Y on endocrine parameters (Table 1)

Neuropeptide Y overexpression in the paraventricular hypothalamus and lateral hypothalamus increased blood leptin concentrations and insulin concentrations to similar extents, which was expected as their amounts of adipose tissues were quite similar. In addition, neuropeptide Y overexpression in the paraventricular hypothalamus also increased blood glucose, corticosterone and adrenocorticotropic hormone concentrations.

Overexpression of agouti-related peptide in the paraventricular hypothalamus and lateral hypothalamus increased leptin concentrations and insulin concentrations. Levels of corticosterone and adrenocorticotropic hormone were not determined in agouti-related peptide overexpressing animals. While insulin concentrations were quite similar

between different groups, there were significant differences between leptin concentrations with agouti-related peptide overexpression in paraventricular hypothalamus and lateral hypothalamus; agouti-related peptide overexpression in the lateral hypothalamus resulted in leptin levels which are comparable with the levels in neuropeptide Y overexpression animals. However, leptin levels in rats which overexpress agouti-related peptide in the paraventricular hypothalamus are 2 fold higher than the levels measured in AAV-LH-AgRP rats, while they have similar amounts of adipose tissue. This suggests that agouti-related peptide in the paraventricular hypothalamus has different effects on leptin secretion than in the lateral hypothalamus. This may be due to differences in innervations to the sympathetic nervous system. lateral hypothalamus and paraventricular hypothalamus are via interneurons connected to adipose tissue (Kreier et al., 2006; Song et al., 2005, 2008) and 60% of these projections from the brain to WAT and BAT co-express melanocortin MC_4 receptor mRNA (Song et al., 2005, 2008). In addition, melanocortin receptor ligands affect SNS activity (Nogueiras et al., 2007) and SNS activity is decreased in obese melanocortin MC_4 receptor knockout mice and humans (Tallam et al., 2005; Greenfield et al., 2009) and i.c.v. administration of melanocortin ligands affected SNS activity. The paraventricular hypothalamus regulates sympathetic activity differently from the lateral hypothalamus. Lesions in the paraventricular hypothalamus decrease sympathetic activity, while lesions in the lateral hypothalamus increase sympathetic activity (Arase et al., 1987; Yoshida et al., 1983; Sakaguchi et al., 1988; Takahashi and Shimazu, 1981; Zhong et al., 2008). The SNS activity is relayed to the periphery via noradrenalin which binds β-adrenoreceptors and *in vitro* studies showed that β receptor agonists decreased leptin secretion by adipocytes (Gettys et al., 1996; Cong et al., 2007). Therefore, overexpression of Agouti-related peptide in the lateral hypothalamus may increase the levels of SNS activity and thereby decrease leptin secretion by adipocytes. In contrast, agouti-related peptide in the paraventricular hypothalamus decreases SNS activity and increases leptin secretion relative to the amount of WAT.

1.7. Effects of agouti-related peptide and neuropeptide Y on peptide expression in arcuate nucleus

Overexpression of neuropeptide Y in the paraventricular hypothalamus and lateral hypothalamus decreased agouti-related peptide mRNA in the arcuate nucleus. In addition, neuropeptide Y overexpression in the lateral hypothalamus also decreases neuropeptide Y mRNA in the arcuate nucleus. In contrast, when agouti-related peptide was overexpressed in paraventricular hypothalamus or lateral hypothalamus, no alterations in pro-opiomelanocortin, neuropeptide Y, agouti-related peptide or suppressor of cytokine signaling 3 mRNA levels were detected.

This is in agreement with a study in which agouti was overexpressed in the paraventricular hypothalamus or lateral hypothalamus and were no changes in neuropeptide Y, pro-opiomelanocortin or agouti-related peptide mRNA compared to controls were found (Kas et al., 2004). In addition, mice which lack melanocortin MC_4 receptor or agouti-related peptide, or mice with ectopic expression of agouti have normal neuropeptide Y and pro-opiomelanocortin mRNA levels in the arcuate nucleus (Qian et al., 2002). Moreover, neuropeptide Y signaling is not altered in melanocortin MC_4 receptor knockout mice, since these mice showed a normal feeding response when injected with neuropeptide Y (Marsh et al., 1999a,b). These data suggest that altered melanocortin signaling cannot be compensated for by neuropeptide Y signaling. In contrast, the melanocortin system appears to be able to compensate for altered neuropeptide Y signaling. Viral mediated overexpression of neuropeptide Y in the lateral hypothalamus or paraventricular hypothalamus, reduced agouti-related peptide mRNA in the arcuate nucleus, while it did not alter pro-opiomelanocortin mRNA (Tiesjema et al., 2007). In addition, neuropeptide Y knockout mice show an increase in agouti-related peptide mRNA (with no changes in pro-opiomelanocortin mRNA) and these mice are more sensitive for effects of Agouti-related peptide (Marsh et al., 1999a,b; Patel et al., 2006). These data implicate that increased neuropeptide Y signaling may be (partially) compensated by an increase in melanocortin signaling.

2. Conclusions

The orexigenic genes, neuropeptide Y and agouti-related peptide, are produced by the same neurons in the arcuate nucleus (Broberger et al., 1998; Baskin et al., 1999) and in several brain areas neuropeptide Y Y_1R and MC_4R co-localize, e.g. paraventricular hypothalamus and central amygdala (Kishi et al., 2005). After binding to their receptors agouti-related peptide and neuropeptide Y both reduce cAMP levels (Gerald et al., 1996; Lu et al., 1994). Thus, one might expect that neuropeptide Y and agouti-related peptide overexpression act similarly at projection sites of the arcuate nucleus to increase food intake and body weight. However this review shows that there are differences in meal patterns, locomotor activity, body temperature and corticosterone levels. In addition, neuropeptide Y signaling may be compensated by melanocortin signaling (since overexpression of neuropeptide Y alters agouti-related peptide mRNA), while neuropeptide Y signaling is not altered by decreased melanocortin signaling.

Agouti-related peptide release in the hypothalamus is probably involved in regulation of satiation and it effects can last for days after a single injection (Hagan et al., 2000). In contrast, in the paraventricular hypothalamus neuropeptide Y appears to be involved in initiation of food intake while in the lateral hypothalamus it is involved in satiation. Thus, neuropeptide Y appears to have multiple functions depending on the area of release and the effects after a single injection last only for hours (Hagan et al., 2000).

The paraventricular hypothalamus and lateral hypothalamus have projections to the hindbrain and melanocortins can alter satiation by interfering with sensitivity for cholecystokinin (Blevins et al., 2009; Fan et al., 2004). Thus, inhibition of melanocortin signaling in neurons of the paraventricular hypothalamus or lateral hypothalamus may decrease satiation in the hindbrain via these projections. The paraventricular hypothalamus has direct projections to the hindbrain which contain oxytocin and corticotropin releasing hormone (Blevins et al., 2009). It would therefore be interesting to study if oxytocin or corticotropin releasing hormone expression is altered after inhibition of melanocortin signaling in the paraventricular hypothalamus, in order to confirm a direct link between paraventricular hypothalamus–hindbrain that can influence meal size in the hindbrain. In addition, it would be interesting to investigate if inhibition of melanocortin signaling in the lateral hypothalamus alters oxytocin or corticotropin releasing hormone expression in the paraventricular hypothalamus via hypothalamic interconnections or if they directly modulate satiation in the hindbrain.

The differences between agouti-related peptide and neuropeptide Y on elements of energy homeostasis may be due to differences in signaling; agouti-related peptide inhibits Gs coupled receptor, while neuropeptide Y activates Gi coupled receptors (Wilson et al., 1999). It was thought that neuropeptide Y and agouti-related peptide activated/inhibited pathways were redundant since neuropeptide Y knockout and agouti-related peptide knockout mice don't have an obvious disturbance of energy balance. However, when agouti-related peptide and neuropeptide Y are injected in the paraventricular hypothalamus they act in a synergistic manner (Wirth and Giraudo, 2000). Thus neuropeptide Y and agouti-related peptide are not redundant. In addition, i.c.v. infusion of agouti-related peptide and neuropeptide Y revealed differences c-Fos activition in extrahypothalamic areas (Hagan et al., 2001). Together, these data underscore that neuropeptide Y and agouti-related peptide exert their orexigenic functions via distinct mechanisms.

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