A Review of Single-Nucleotide Polymorphisms (SNPs) in Orexigenic Neuropeptides Targeting G-Protein Coupled Receptors (GPCRs)

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Abstract

Many physiological pathways are involved in appetite, food intake, and the maintenance of energy homeostasis. In particular, neuropeptides within the central nervous system have been demonstrated to be critical signaling molecules for modulating appetite. Both anorexigenic (appetite-decreasing) and orexigenic (appetite-stimulating) neuropeptides have been described. The biological effects of these neuropeptides can be observed following central administration in animal models. This review will focus on single nucleotide polymorphisms (SNPs) in six orexigenic neuropeptides: agouti-related protein (AGRP), galanin, melanin concentrating hormone (MCH), neuropeptide Y (NPY), orexin A, and orexin B. Following a brief summary of the neuropeptides and their orexigenic activities, reports associating SNPs within the orexigenic neuropeptides to energy homeostasis, food intake, obesity, and BMI in humans will be reviewed. Additionally, the NIH tool Variation Viewer was utilized to identify missense SNPs within the mature, biologically active neuropeptide sequences. For SNPs found through Variation Viewer, a concise discussion on relevant pharmacological structure-activity relationship (SAR) studies for select SNPs is included. This review is meant to update reported orexigenic neuropeptides SNPs and to demonstrate the potential utility of genomic sequence databases for finding SNPs that may result in altered receptor signaling for neuropeptide pathways associated with appetite.

Graphical Abstract

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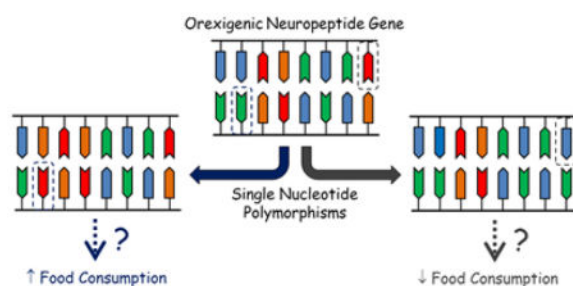
Keywords

Single-nucleotide polymorphisms; SNP; agouti-related protein; galanin; melanocyte concentrating hormone; neuropeptide Y; orexin A; orexin B; polypharmacology; pharmacogenomics; GPCR; hormone; peptide

Introduction

With an estimated 36.5% of US adults being obese from 2011–2014,[1] and 39% of the global adult population being overweight and 13% obese,[2] obesity and accompanying co-morbidities continue to be a problem. While monogenetic origins of obesity exist, including polymorphisms in the leptin receptor[3] and melanocortin-4 receptor,[4–5] the interactions of many different genetic, behavioral, and environmental factors all contribute to the dysregulation of energy homeostasis that can result in obesity, as previously reviewed.[6] The heritability of BMI has been estimated to be 0.77 from twin studies,[7] and adoption studies have suggested that body weight class (thin, median, overweight, obese) of an adoptee is correlated to the BMI of the biological parent and not the adoptive parent,[8] indicating genetics are important in the risk of developing obesity. Investigating biological circuits associated with appetite may therefore be important in understanding the etiology of obesity and identifying novel targets for development of therapeutics to help modulate body weight.

Numerous physiological pathways, involving peripheral and central signals, have been implicated in the maintenance of energy homeostasis. Within the central nervous system, peptides have been demonstrated to be important signaling molecules that can increase or decrease appetite (orexigenic or anorexigenic peptides, respectively). While peptides involved in energy homeostasis may be synthesized in the periphery and act centrally (including leptin),[9] this review will focus on neuropeptides, defining neuropeptides as "small proteinaceous substances produced and released by neurons through the regulated secretory route and acting on neural substrates."[10]

Specifically, orexigenic neuropeptides will be examined that have been associated with an increase in food consumption in animal models when the exogenous neuropeptide is centrally administered. The orexigenic neuropeptides included in this review are agouti-related protein (AGRP), galanin, melanin concentrating hormone (MCH), neuropeptide Y, orexin A, and orexin B. The pharmacological activity and orexigenic activities of these neuropeptides in animal models will be summarized. Due to the observed orexigenic effects, dysregulation of these neuropeptides and their signaling may be hypothesized to result in

altered food consumption. Decreased signaling may lower hunger (resulting in leanness) while increased signaling may stimulate appetite (resulting in weight gain and obesity). Mutations or polymorphisms within the orexigenic genes that modulate the expression level, receptor affinity, or functional activity of the neuropeptides may alter the resulting biological signaling and observed phenotype.

This review will highlight single nucleotide polymorphisms (SNPs; a change or mutation in one DNA base pair within a gene sequence) associated with the orexigenic neuropeptides that have been postulated to result in altered energy homeostasis, including inherited leanness and obesity. These SNPs were identified by genotyping individuals and identifying SNPs within target genes that are present or enriched in an experimental cohort (an obese subset, for example) but not the control population, as an updated reference to prior reviews. [11–12] The location of the SNPs within the gene structure (promoter, signal sequence, intron, exon) are identified.

In addition to the reported neuropeptide SNPs in published association studies, this review will identify SNPs deposited into the NCBI database for the neuropeptide sequences and observed using the Variation Viewer tool. For this review, the database was accessed in March 2018. The continued submissions of new genomic data will generate additional SNPs not present in this review, making this publication a snapshot of deposited SNPs up to March 2018. For this section, only SNPs located within the mature neuropeptide sequences are included to limit SNPs to the biologically active amino acid sequence. Additionally, only missense polymorphisms (polymorphisms that alter the amino acid sequence of the mature neuropeptide) are included. These limitations are used so the deposited SNPs could be described in the context of prior structure-activity relationship (SAR) studies correlating the structure of the neuropeptides with pharmacological effects. Such observations highlight only a small part of the known SAR of the neuropeptides. Accordingly, a comprehensive review for each neuropeptide's known pharmacology is not included in this review, but key reports are provided as an introduction to SAR studies for deposited SNPs that may potentially alter receptor binding and function. While polymorphisms in several of the orexigenic neuropeptide receptors have been linked to altered energy homeostasis in humans, including the melanocortin-3 and melanortin-4 receptors (as previously reviewed)[13–14] this review will focus on SNPs located in the orexigenic neuropeptides. Additionally, although several anorexigenic neuropeptide polymorphisms have been associated with altered receptor pharmacology (including α-MSH and β-MSH),[15–18] this review will only highlight orexigenic SNPs.

Orexigenic Neuropeptides

Agouti-Related Protein

First reported in 1997, human agouti-related protein (hAGRP) is transcribed as a 132 amino acid protein.[19–21] One of two currently known endogenous antagonists to G-protein coupled receptors (GPCRs), AGRP has been shown to possess nanomolar antagonist potency at the melanocortin-3 receptor and melanocortin-4 receptors (MC3R and MC4R),[19–20] and possess inverse agonist activity at the MC4R.[22–23] Transgenic mice overexpressing AGRP weighed significantly more than wild-type littermates,[20, 24] and intracerebroventricular (ICV)

administration of recombinant AGRP induced a dose-dependent food intake increase in rats,[25] demonstrating an orexigenic effect for AGRP. Further work hypothesized that the C-terminal domain of AGRP [AGRP(83-132); Figure 1] is the active form *in vivo* following processing by proprotein convertase 1.[26] Central administration of the C-terminal domain of AGRP via ICV[26–29] or intrathecal[30] (IT) administration has been demonstrated to increase food intake in rodents. A dose-dependent increase in food intake is observed when the C-terminal domain of AGRP is administered in MC3R knockout (KO) or MC4R KO mice, suggesting pharmacology of both receptors is important for modulating the feeding response.[27] While excess AGRP results in increased feeding, AGRP knockout mice maintain similar weight and feeding behaviors to wildtype mice,[31] although an age-dependent leaner phenotype beginning at 6 months has been observed.[32] A key Arg-Phe-Phe tripeptide sequence has been identified as critical for binding to the MC3R and MC4R.[33] Solution NMR structures of the C-terminal domain of AGRP or a truncated "mini-AGRP" suggest this tripeptide sequence is located on an exposed beta-hairpin loop.[34–36] The charge of exogenously administered AGRP has been correlated to the feeding response in mice, with more basic (positively) charged residues outside the melanocortin binding motif resulting in increased food intake and higher changes in body mass.[37] This correlation between charge and activity has been hypothesized to be due to AGRP non-specifically binding to negatively charged heparan sulfate proteoglycans (including syndecan-3), helping to concentrate AGRP at the cell surface near the MC3R/MC4R.[38]

Single nucleotide polymorphisms in the promoter, introns, and exons of AGRP have been investigated for potential linkage to body weight and obesity. As previously reviewed in 2008, polymorphisms in the promoter of AGRP (rs5030981, rs8047574. and rs34018897) were associated with reduced risk for obesity and type 2 diabetes (rs5030981 and rs8047574) and a potential predisposition for decreased resting metabolic rate/increased fat mass (rs34018897).[11] Another study of rs5030981 also reported a lower BMI in females and lower rates of type 2 diabetes in males of West African descent.[39] For a polymorphism in the second intron of AGRP (rs11575892) found from screening 95 patients with severe obesity, heterozygotes at this position possessed significantly higher BMI compared to controls (30.97 versus 27.92, respectively).[40] In this same study, a SNP within the translated region of AGRP (rs5030980, Ala67Thr) was not associated with BMI.[40] As previously reviewed, this polymorphism (rs5030980) has previously been associated with anorexia nervosa, inherited leanness, and resistance to late on-set obesity.[11] When examining a genetic variants of obesity candidate genes in a European American population, the rs5030980 SNP was found to be modestly associated with BMI.[41] Another report in a Dutch population suggested that the Thr allele of rs5030980 was associated with an increased BMI in males, but not females.[42] Thus, this allele has been linked to decreased BMI, no association with body weight, and increased BMI, depending on the population examined. Analysis of the rs5030980 polymorphism in an *in vitro* expression system did not clarify these observations, as there were no differences between the Ala and Thr polymorphisms for AGRP cellular distribution or processing, antagonism of α-MSH-mediated stimulation of the MC4R, or induction of rat food intake.[43]

While no SNPs located within the *in vivo* active form of AGRP [AGRP(83-132), Figure 1] have been associated with altered metabolism in humans, some SNPs deposited in the NIH

database may be important for functional activity. Within the C-terminal domain of AGRP, there are 10 Cys forming 5 disulfide bonds, resulting in a highly structured peptide as determined by NMR.[34–36] For SNPs found within the NIH Variation Viewer, 5 Arg to Cys polymorphisms have been reported [Arg85 (rs765334298), Arg86 (rs964545025), Arg111 (rs1012110755), Arg120 (rs769073853), and Arg131 (rs201485380); Figure 1]. Four Cys residues that form the disulfide bonds in the native protein also possess SNPs [Cys108 (rs1287345613), Cys117 (rs767874407), Cys119 (rs1392000625), and Cys129 (rs1271631561)]. These polymorphisms introduce or eliminate a Cys residue, potentially disrupting the native disulfide bonds and altering the secondary structure which may decrease affinity or functional activity. There are also 8 positions that possess polymorphisms that decrease the net positive charge of AGRP at physiological pH [Arg85 (rs765337298), Arg86 (rs964545025 & rs759822430), Arg89 (rs773694020), His91 (rs772682095 & rs956734327), Arg111 (rs1012110755), Arg120 (rs769073853), Gly123 (rs1191172042), and Arg131 (rs201485380)], and 7 positions where polymorphisms increase the net positive charge [Gly96 (rs768146316), Gln97 (rs1473841047), Gln98 (rs1238154717), Thr107 (755684740), Tyr118 (rs762405425), Cys119 (1392000625) and Met126 (rs750837166)], indicating polymorphisms that might alter AGRP association with heparan sulfate proteoglycans and affect food intake.[37–38] There are an additional seven polymorphisms within the hypothesized beta-hairpin active loop of AGRP [Arg111Cys/Gly (rs1012110755), Arg111His (rs199927717), Phe112Tyr (rs200972106), Ala115Val (rs773319622), Ala115Thr (rs1321944438) and Phe116Leu (1414719119)], of which four (Arg111 and Phe112 polymorphisms) are located within the purported tripeptide Arg-Phe-Phe active sequence of AGRP. The equivalent substitution to the Phe116Leu polymorphism was assayed in an octapeptide AGRP macrocycle scaffold, and was found to decrease potency 15-fold relative to the native Phe,[44] suggesting polymorphisms in this purported loop may decrease potency relative to the native sequence.

Galanin

The galanin neuropeptide was first identified from a porcine intestinal extract in 1983.[45] Named for the N-terminal glycine and C-terminal alanine, the 123 residue preprogalanin is processed into the 30 amino acid galanin neuropeptide in humans (Figure 2).[46] Unlike other species, the C-terminal of galanin in humans is not carboxyamidated.[46] As previously reviewed, human galanin possesses sub-nanomolar affinity at the galanin-1 receptor (GalR1), sub-nanomolar to nanomolar affinity at the galanin-2 receptor (GalR2), and tens of nanomolar affinity at the galanin-3 receptor (Gal3R).[47] Administration of galanin into the paraventricular nucleus in male Sprague Dawley rats resulted in a dose dependent increase in food intake over saline control at 30 and 60 min, with the maximal effect at 1 nmol galanin, demonstrating the orexigenic activity of the galanin neuropeptide.[48] While wildtype, galanin KO, and galanin overexpressing mice maintain similar levels of food intake on a standard diet,[49–50] differences are observed when a high-fat diet is utilized. Following introduction of a high fat diet (45% fat), wildtype mice consumed more and gained more body weight than galanin KO mice.[50] In mice maintained on a standard diet and induced to drink 15% ethanol, galanin overexpressing mice consumed more following the introduction of a high fat diet compared to wildtype mice, with a greater increase noted in female mice.[51] These data may suggest that galanin has a role in dietary fat intake. While

several preprogalanin SNPs have been identified, currently no SNPs have been definitely correlated with altered BMI or obesity in humans.[52–53]

While no galanin SNPs are associated with the dysregulation of energy homeostasis, select SNPs reported in the Variation Viewer database may affect the binding affinity or functional activation of galanin with the galanin receptors. Work with porcine galanin suggested the N-terminal domain of galanin has similar affinity and functional activity compared to the full length peptide.[54–58] The C-terminal domain may help prevent the degradation of galanin since the N-terminal fragment Gal(1-16) (numbering based upon the N-terminal Gly residue set to 1) possesses a shorter half-life compared to the full length porcine galanin.[59–60] Thus, critical residues for ligand-receptor function may be located in the N-terminal domain 16 residues of galanin, of which 12 positions possess a SNP in humans [Gly1 (rs1380829651), Trp2 (rs1208503949), Thr3 (rs201520007), Leu4 (rs528520052), Asn5 (rs772658511), Ser6 (rs746516038), Ala7 (rs770751421 and rs1057517661), Gly8 (rs1323568387), Gly12 (rs530344730 and rs1460598118), Pro 13 (rs1251622772 and rs1421258394), Ala15 (rs374472664 and rs1402134762), and Val16 (rs541536020), Figure 2]. The first 15 residues of human and porcine galanin are identical, with a conservative substitution at position 16 (Val in humans and Ile in pigs). Interestingly, the Val16Ile is a human polymorphism (rs541536020), indicating pharmacological characterization of the porcine galanin may be representative of this human SNP. An Ala positional scan on the N-terminal Gal(1-16) fragment of the porcine galanin demonstrated that Ala substitution at the Trp2, Asn5, Tyr9, Leu10, Leu11, and Gly 12 positions decreased the ability to displace radiolabel ^{125}I-Gal >100-fold in membrane binding studies from homogenized rat hypothalami, with complete loss of affinity for the Trp2Ala substitution at concentrations up to 100 μM.[61] Three of these positions possess SNPs in the human galanin peptide, Trp2Leu (rs1208503949), Asn5Lys (rs772658511) and Gly12Asp/Ala (rs530344730), and may represent SNPs that modulate the affinity of human galanin for the galanin receptors.

Melanin Concentrating Hormone

The melanin concentrating hormone (MCH) was first isolated in 1983 from salmon as a peptide hypothesized to be cyclized through a disulfide bond,[62] and subsequently from rat hypothalami.[63] In humans, the 19 residue MCH is processed from a 165 residue preprohormone (Figure 3),[64] and has nanomolar affinity for two receptors, the melanin concentrating hormone receptor 1 (MCHR1) and melanin concentrating hormone receptor 2 (MCHR2), as previously reviewed.[65] Many studies have indicated that ICV administration of MCH increases food intake in rodents.[66–68] Unlike other neuropeptides reviewed herein, MCH KO mice have an altered metabolic phenotype. Compared to wildtype mice, both male and female MCH KO mice are hypophagic (lower food intake) and possess a lower body weight, as originally reported by Shimada *et al.*[69] For MCH over-expressing transgenic mice, differential weight gain compared to wildtype mice was dependent on the genetic background of the mouse and gene copy number. Homozygous transgenic mice and a high fat diet were required to result in increased weight for mice on of a FVB genetic background, while heterozygotes on standard chow demonstrated a 10% increase in body weight for mice from a C57BL/6J background.[70] All these data support an orexigenic role for the MCH neuropeptide. While to date no studies have associated MCH SNPs with

altered weight gain or BMI in a human general population, one SNP (rs7973796) outside the active fragment has been associated with increased BMI in schizophrenic patients taking olanzapine,[71] a finding that was also presented at the 4th Biennial Schizophrenia International Research Conference.[72]

Despite the lack of MCH SNP association to altered energy homeostasis in humans, SNPs within the active MCH neuropeptide may affect receptor affinity and the ligand-receptor interface. The Cys7Tyr/Phe polymorphism (rs761128374; numbering based upon the N-terminal Asp residue set to 1) results in loss of the disulfide bond. Previous work on replacing the two Cys with Ala[73] or Ser[74] resulted in ligands lacking agonist potency at 10,000 and 1,000 nM, respectively, indicating the cyclizing disulfide bond is critical for agonist activity and loss of the disulfide bond might be expected to decrease agonist potency. In an Ala positional scan of the hMCH peptide, substitution of Ala at the Arg6, Arg11, and Tyr13 positions decreased binding affinity >10-fold and functional activity >7-fold at both the MCH1R and MCH2R.[73] These data suggest that the Arg6Ile (rs781675279), Arg11Lys (rs905199758), and Tyr13Asp (rs774549475) SNPs may negatively impact ligand-receptor interactions. In another report, a Lys residue was used to replace the Arg11, corresponding to the Arg11Lys peptide (SNP rs905199758). When characterized at the MCHR1, the Arg11Lys peptide possessed decreased binding affinity (IC_{50} = 197 versus 1.5 nM) and functional potency (EC_{50} = 5100 versus 12 nM), and only stimulated the receptor to 67% maximal signal compared to the native sequence.[75] In a minimized cyclic scaffold, (H-Arg-c[Cys-Met-Lue-Gly-Xxx-Val-Thr-Arg-Pro-Cys]-Trp-OH, where Xxx corresponds to Arg11 in the native sequence), the equivalent Arg to Lys substitution did not possess activity up to micromolar concentrations.[74] These data are pharmacological characterization of an endogenous MCH SNP (rs905199758) and demonstrate >100-fold decreased affinity and functional activity, suggesting decreased agonist affinity and potency for this SNP.

Neuropeptide Y

The 36-residue neuropeptide Y (NPY) was first identified and sequenced in 1982 from porcine brain tissue.[76–77] Translated as a 97 residue prepropeptide,[78] cells expressing proprotein convertase 1, proprotein convertase 2, or both efficiently produce NPY from pro-NPY following removal of the signal sequence (the mature sequence can be found in Figure 4).[79] The C-terminal of NPY is carboxyamidated, and NPY(1-36) may be further processed by aminopeptidase P[80–81] and dipeptidyl peptidase IV[80] into NPY(2-36) and NPY(3-36), respectively. There are four neuropeptide Y receptors in humans (Y1R, Y2R, Y4R and Y5R), with NPY being a hypothesized endogenous ligand at the Y1R, Y2R, and Y5R, as previously reviewed.[82] Central administration of NPY increased cumulative food intake and decreased time latency to feeding response in rats, as first demonstrated in 1984.[83–84] In CD-1 mice, central administration of NPY(1-36), NPY(2-36), and NPY(3-36) increased food consumption, while NPY(13-36) did not stimulate higher intake compared to vehicle.[85] Analogous to AGRP KO and galanin KO mice, NPY KO mice possess similar food intake and body weight to wild-type mice.[86] Normal body weight is also maintained in NPY/AGRP double KO mice.[31] The body weight of transgenic mice overexpressing NPY was also similar to the weight of wildtype mice on a standard diet,[87–89] although NPY-overexpressing mice consuming a high sucrose diet weighed more than wildtype controls.[89]

Correlational studies have examined potential SNPs in the promoter, introns, signal sequence, translated polypeptide chain, and 5′ untranslated region in the NPY gene since a 2003 review.[12] Within the promoter region of NPY, the rs17149106 SNP was associated with an increased prevalence of obesity in American health care professionals.[90] Another NPY promoter SNP, rs16147, has a mixed association. A positive association with obesity was found in Malaysian[91] and Spanish[92] youth along with a higher BMI from infants to adulthood in a German population,[93] but did not increase the prevalence in obesity in an American health care population[90] nor significantly alter BMI in a German cohort.[94] Two SNPs in intron 1 (rs16478) and intron 2 (rs16135) of the NPY gene transcript were not associated with obesity while a SNP in intron 3 (rs16131) was positively associated with obesity in a Spanish youth population.[92] Within the translated polypeptide chain, the rs16139 SNP results in a Leu to Pro substitution in the signaling sequence.[95] This polymorphism has been associated with a higher BMI in a male Dutch cohort,[42] in a non-obese Swedish population,[96] and in a female type-1 diabetic Finnish population,[97] and an increased prevalence of obesity in a survey of US male health professionals[90] and in an Iranian cohort with metabolic syndrome.[98] However, other studies reported the rs16139 polymorphism to not be associated with weight gain in Finnish youth,[99] does not reproducibly associate with BMI in several populations,[100] and is not associated with obesity in an South Indian cohort.[101] A 2015 meta-analysis of 6 case-controlled studies of the rs16139 SNP indicated that the Leu to Pro polymorphism is associated with an increased risk of obesity.[91] Two silent mutations (rs9785023 and rs5574), one of which located within the active form of NPY (rs9785023) have been investigated, although neither SNP was associated with obesity.[90–91, 100–101] One SNP found in the NPY transcript, outside of the active NPY neuropeptide (rs931762615; Val to Asp), was reported in an obese patient and her obese father, and was not found in a control population.[102] One additional SNP at the 5′ untranslated domain of the NPY transcript was also not associated with obesity in a South Indian population.[101]

Numerous missense mutations have been recorded in the human NPY active polypeptide sequence in the NCBI database for Variation Viewer (Figure 4). One polymorphism, Met17Leu (rs368302969, numbering based upon the N-terminal Tyr set to 1) would generate a peptide with the identical sequence to the initially reported porcine NPY.[76] The porcine NPY has been utilized in alanine and D-amino acid positional scans,[103–107] which have indicated that the C-terminal portion of NPY is critical for ligand binding and functional activity. A porcine NPY(1-36) peptide with the Tyr36Phe substitution (corresponding to the rs1369007793 SNP with an additional Met17Leu substitution) decreased affinity at the hY1R and hY2R 13- and 5-fold, respectively (a Tyr36His substitution decreased affinity >2,300- and >220-fold).[103] In another porcine analog, the Pro8Ala (corresponding to SNP rs999271326) decreased affinity 160- and 9-fold to SK-N-MC and SMS-KAN cells (presumed to express the hY1R and hY2R), and decreased efficacy 54-fold relative to NPY in a rat jejunum bioassay.[103, 105] While the Tyr36Phe and Pro8Ala are representative of human SNPs, these porcine NPY sequences possessed an additional Met17Leu substitution, a double polymorphic result that may not be representative in humans. Additionally, in the human NPY sequence the Pro5Ala substitution (rs1253206041) decreased affinity 1,100- and 300-fold to SK-N-MC and SMS-KAN cells and possessed 16-fold decreased potency in

the rat jejunum assay compared to NPY(1-36), while the Pro13Ala substitution (rs1167638996) decreased affinity 375-fold to SK-N-MC cells, possessed equipotent affinity to SMS-KAN cells, and 17-fold decreased potency in the rat jejunum assay compared to NPY(1-36).[103, 105] Also utilizing the human NPY(1-36) sequence, the Tyr20Phe substitution (corresponding to SNP rs753592250) was previously characterized to examine the effects of removing the hydroxyl group from aromatic ring in the human NPY.[108] The Tyr20Phe peptide significantly decreased affinity in rat CNS membranes (IC_{50} = 10.1 nM versus 3.8 nM for hNPY) and potency in a rat van deferens bioassay (EC_{50} = 234 nM versus 44.3 nM for hNPY).[108] These data represent NPY SNPs that have been pharmacologically characterized to possess decreased affinity and/or functional activity, which may be relevant in altering NPY signaling for food consumption.

Orexin A and Orexin B

The orexin (hypocretin) system was reported by two independent groups in 1998.[109–110] One group noted the orexigenic (increased food intake) response upon ICV administration of the endogenous ligands, labeling the neuropeptides and receptor family the orexins.[110] The other group reported expression of the endogenous peptide preprohormone exclusively in the hypothalamus and shared amino acid sequence identity with the secretin hormone, naming the discovered receptor system the hypocretins.[109] While both names have been used interchangeably, one nomenclature recommendation has been to name the gene and mRNA products as hypocretins while the precursor peptide, process peptides, and protein receptor products as orexins,[111] a scheme that will be following in the present review. The 131-residue human preproorexin precursor peptide is processed into two endogenous orexin agonists, the 33-residue orexin A and 28-residue orexin B (Figures 5 and 6).[112] The C-terminal of both orexin A and orexin B have been hypothesized to be carboxyamidated, while the N-terminal Gln of orexin A has been purported to be cyclized into a pyroglutamyl residue.[110, 112] Orexin A also possesses 4 Cys residues that form two disulfide bonds, the topology of which was determined to be Cys6-Cys12 and Cys7-Cys14 by synthesizing the different possible disulfide pairings.[110] Orexin A and orexin B are the endogenous agonists for two receptors, the orexin-1 receptor (OX1R) and the orexin-2 receptor (OX2R).[110] Orexin A was reported to possess similar affinity for the OX1R and OX2R (20 nM and 38 nM), while orexin B was approximately 10-fold selective for the OX2R over the OX1R (36 nM and 420 nM).[110] The ligands possessed a similar functional response (orexin A equipotent at the OX1R and OX2R; orexin B 10-fold more potent at the OX2R over the OX1R) compared to the affinity data when utilizing an assay to measure intracellular Ca^{2+} concentrations.[110, 113]

One of the original papers describing the orexins noted that icv administration of orexin A or orexin B dose-dependently increased food consumption in rats when administered in the early light phase.[110] Increased food intake for orexin A administered in the early light phase has been replicated in rats,[114–115] although the response following icv administration of orexin B have been reported to both increase[114] and have no effect[115] on food intake. Orexin KO mice do not possess significantly altered body weight compared to wildtype controls,[116] although they possess a phenotype similar to human narcolepsy.[117] Genetic ablation in mice of neurons producing the preproorexin peptide also produce a narcolepsy-like phenotype,[118]

which is accompanied by a late-onset obesity dependent on the background of the mice. [116, 118] These mice develop obesity despite being hypophagic, and were reported to have decreased spontaneous motor activity.[118] Overexpression of the orexins prevents obesity in mice fed a high-fat diet compared to a low-fat diet, where male and female mice overexpressing the preproorexin transcript possess similar body weights to low-fat fed wildtype mice, up to 30 weeks.[119] While SNPs have been reported for the preproorexin peptide, currently no SNPs have been directly linked to human obesity.[120]

Within Variation Viewer, SNPs are present in nearly half of the amino acids of orexin A (15 of 36 residues, Figure 5). A systematic truncation study of orexin A indicated that the N-terminal 5 amino acids may be removed with approximately 10-fold decreased potency at both the OX1R and OX2R,[121] suggesting the Pro4Ala, Asp5Asn, and Asp5Glu (rs1373951353, rs754595506 and rs754232977, respectively; numbering with the N-terminal pyro-Gln residue set to 1) SNPs may have minimal effect on the pharmacological activity of the neuropeptide. An Ala positional scan of the C-terminal domain of orexin A [orexin A(15-33)] indicated that among other residues, Ala substitution at the Leu20, Gly29, Ile30, Thr32 and Leu33 positions decreased the agonist response at the OX1R, with partial to no receptor activation at 10 μM concentrations (37%, 15%, inactive, 39%, and 18%, respectfully).[122] Similar results were reported with a different orexin A fragment [orexin A(17-33)], with <50% receptor activation using 10 μM concentrations at both the OX1R and OX2R when Gly29, Ile30, Thr32, and Leu33 were substituted with Ala.[123] These positions correspond to human SNPs [Leu20Arg (rs1478595312), Gly29Ser (rs767907538), Ile30Ser (rs1292852639), Thr32Arg(1415661572), and Leu33Met (rs763215233)] which may negatively impact orexin A potency. A Gly24Cys SNP (rs1202994832) introduces an unpaired Cys that can disrupt the native disulfide bonding pattern, potentially disrupting the overall fold of orexin A that may affect affinity and functional activity.

Within the Variation Viewer database, 19 of 28 amino acids of orexin B possess SNPs. Truncation of the N-terminal 5 residues from human orexin B [orexin B(6-28), numbering with the N-terminal Arg residue set to 1] was shown to be equipotent compared to the full length orexin B peptide at both the OX1R and OX2R,[121] suggesting that polymorphisms at the Arg1, Gly3, Pro4, and Pro5 positions may be tolerated (corresponding to SNPs rs1466937991, rs777822783, rs778354341, rs754648418, rs753435149, and rs1206882322). An Ala positional scan of orexin B supports the truncation studies as substitution of the Arg1, Gly3, Pro4, and Pro5 positions with Ala had <10-fold effect on potency at both the OX1R and OX2R.[124] Two of the SNPs in the Variation Viewer database are Ala substitutions [Pro4Ala (rs778354341) and Gly9Ala (rs558579797)] were characterized in the Ala positional scan of orexin B, decreasing potency <4-fold at both the OX1R and OX2R.[124] These data would suggest that these polymorphisms do not negatively affect ligand potency. Similar to orexin A, the C-terminal domain of orexin B has been shown to be important for receptor potency. Removal of the Met28 [orexin B(1-27)] results in a peptide that does not possess agonist activity at either OX1R or OX2R at up to 1 μM concentrations, [121] while Ala substitution at positions 24–28 of orexin B decreases potency by >100-fold relative to the full length peptide at both OX1R and OX2R.[124] These data suggest that the Leu26Pro (rs751706776), Thr27Ile (rs1180653330), Met28Val (rs1277187932), Met28Ile (rs1164006314), and Met28Thr (rs896739180) SNPs may negatively impact ligand potency,

though a Pro positional scan on a fragment of orexin B [orexin B(6-28)] suggested that Pro substitution at the Leu26 position was tolerated.[121]

Conclusions

This reviewed focused on human SNPs in six neuropeptides that have been shown to increase food intake in animal models. Two of the neuropeptides (AGRP and NPY) possess SNPs associated with altered energy homeostasis, including increased BMI and risk of developing obesity. Many additional SNPs have been deposited into the NIH database for the active sequences of AGRP and NPY, as well as the other orexigenic neuropeptides, as highlighted in Figures 1–6. Even more SNPs in the prepropeptides, introns, promoter regions, and corresponding receptors have been deposited, as well as nonsense and frameshift polymorphisms within the active neuropeptide sequences that were not included in this review. Since the majority of the deposited SNPs have not been pharmacologically characterized, the signaling effects of these SNPs are unknown. Additionally, because these SNPs have not been correlated with a phenotype, some caution in interpreting the SNPs is warranted. The accuracy of the deposited SNPs and prevalence in a population are questions that might limit the utility of these data. The majority of the SNPs may also not possess any altered pharmacology, and may not be associated with any phenotypic changes.

With the continued pursuit of personalized medicine and inexpensive sequencing technologies, more polymorphisms will be discovered and reported. Knowing the pharmacological activity of rare polymorphisms may aid the treatment of individuals. In 2016, the MC4R synthetic agonist setmelanotide was used to sustain weight loss in two of the three known individuals globally with proopiomelanocortin deficiency (patients who do not express the endogenous agonist ligands for the melanocortin receptors).[125] As additional SNPs in orexigenic peptides, anorexigenic peptides, and their receptor partners are discovered and pharmacologically assessed for altered signaling, it may be possible to envision similar treatments for individuals with aberrant signaling in pathways associated with appetite and energy homeostasis.

Acknowledgments

Abbreviations used

AGRP	agouti-related protein
BMI	body-mass index
GALR	galanin receptor
GPCR	G protein-coupled receptor
ICV	intracerebroventricular

IT	intrathecal
KO	knockout
MCH	melanin concentrating hormone
MCHR	melanin concentrating hormone receptor
MCR	melanocortin receptor
NPY	neuropeptide Y
OXR	orexin receptor
SAR	structure-activity relationship
SNP	single nucleotide polymorphism

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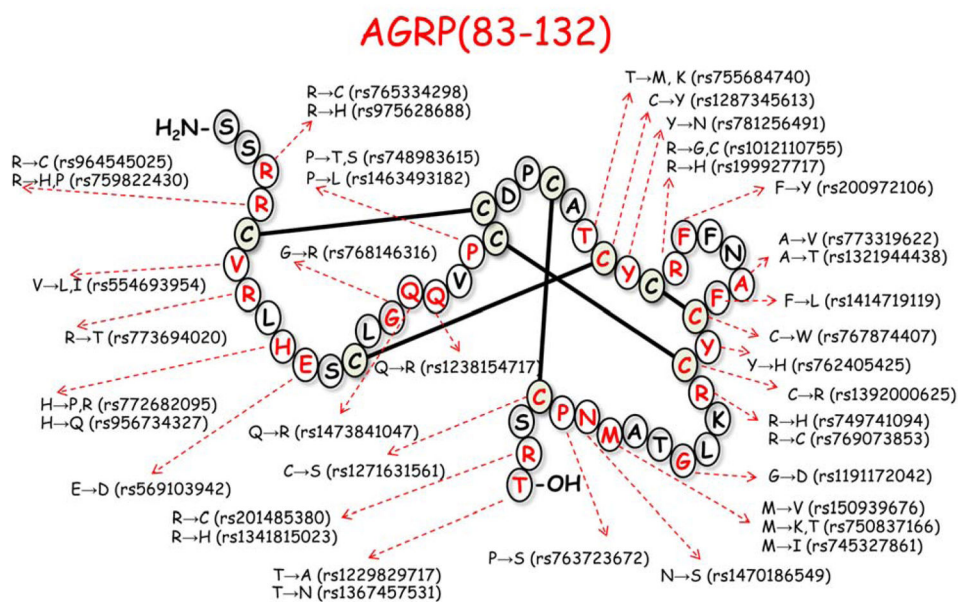
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Figure 1.
The amino acid sequence of AGRP(83-132). Missense SNPs are indicated in red, with the rs accession number and reported variant amino acids. The disulfide connectivity is indicated by black bars.
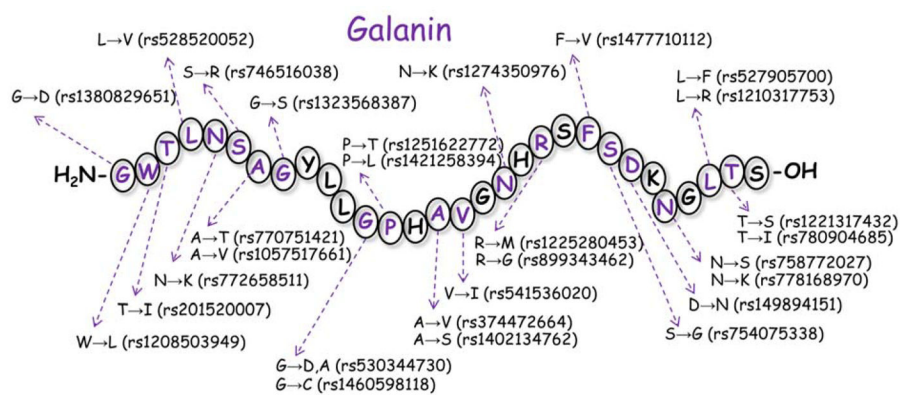
Figure 2.
The amino acid sequence of galanin. Missense SNPs are indicated in purple, with the rs accession number and reported variant amino acids.
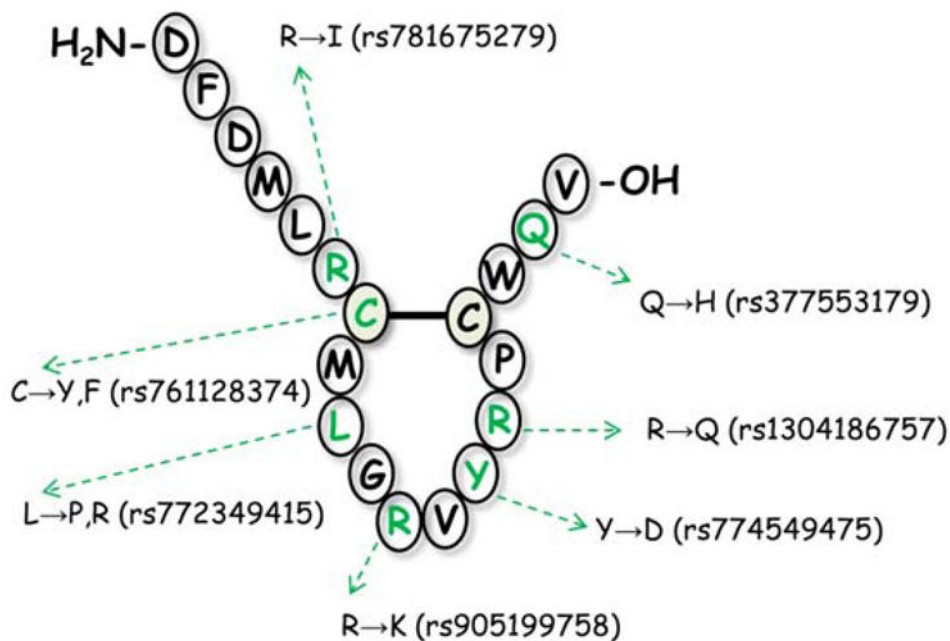
Melanin Concentrating Hormone

Figure 3.
The amino acid sequence of the melanin concentrating hormone (MCH). Missense SNPs are indicated in green, with the rs accession number and reported variant amino acids. The disulfide bond is indicated by the black bar.
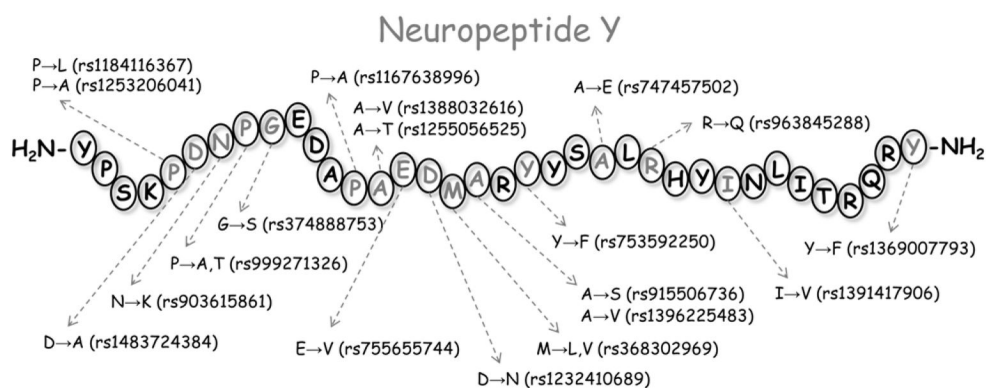
Neuropeptide Y

Figure 4.
The amino acid sequence of neuropeptide Y (NPY). Missense SNPs are indicated in grey, with the rs accession number and reported variant amino acids.
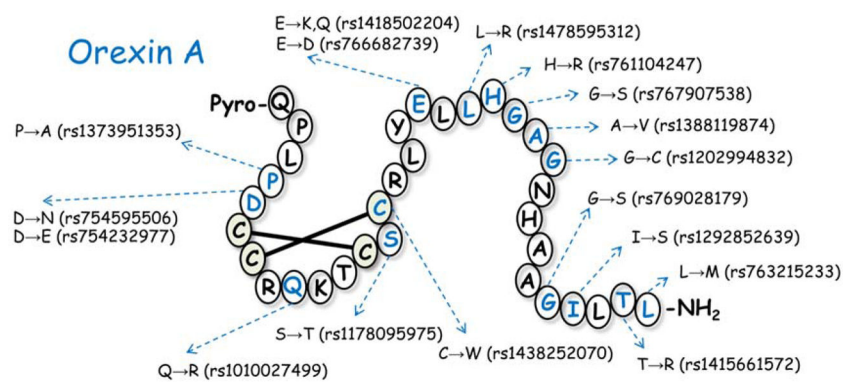
Figure 5.

The amino acid sequence of orexin A. Missense SNPs are indicated in blue, with the rs accession number and reported variant amino acids. The disulfide bonds are indicated by black bars.
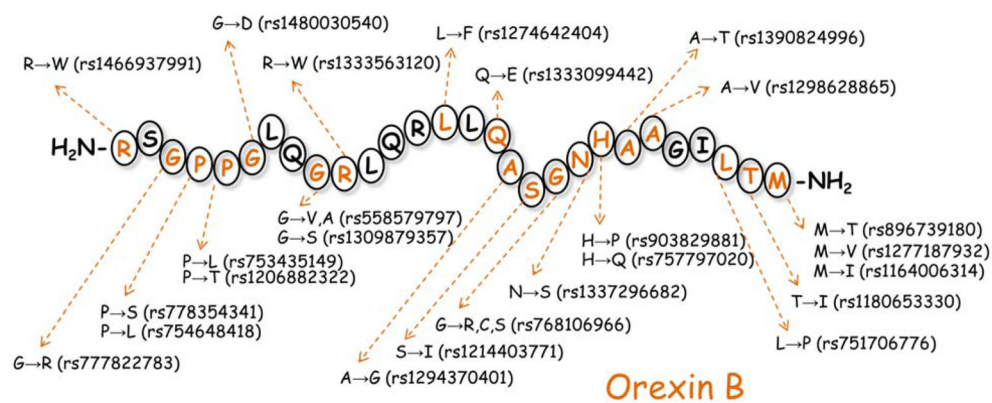
Figure 6.
The amino acid sequence of orexin B. Missense SNPs are indicated in orange, with the rs accession number and reported variant amino acids.