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REVIEW ARTICLE

Maternal stress and diet may influence affective behavior and stress-response in offspring via epigenetic regulation of central peptidergic function

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Abstract

It has been shown that maternal stress and malnutrition, or experience of other adverse events, during the perinatal period may alter susceptibility in the adult offspring in a time-of-exposure dependent manner. The mechanism underlying this may be epigenetic in nature.

Here, we summarize some recent findings on the effects on gene-regulation following maternal malnutrition, focusing on epigenetic regulation of peptidergic activity. Numerous neuropeptides within the central nervous system are crucial components in regulation of homeostatic energy-balance, as well as affective health (i.e. health events related to affective disorders, psychiatric disorders also referred to as mood disorders). It is becoming evident that expression, and function, of these neuropeptides can be regulated via epigenetic mechanisms during fetal development, thereby contributing to the development of the adult phenotype and, possibly, modulating disease susceptibility. Here, we focus on two such neuropeptides, neuropeptide Y (NPY) and corticotropin-releasing hormone (CRH), both involved in regulation of endocrine function, energy homeostasis, as well as affective health. While a number of published studies indicate the involvement of epigenetic mechanisms in CRH-dependent regulation of the offspring adult phenotype, NPY has been much less studied in this context and needs further work.

Key words: neuropeptides; methylation; affective disorders; malnutrition; stress; perinatal

Introduction

Early life adverse events, including malnutrition as well as economic, social, and psychological stress, have been shown to be associated with assorted, predominantly poor, health outcomes and altered, predominantly increased, disease risk in adulthood [1–4]. It is well established that the perinatal period is a crucial time-period for the programming of the adult phenotype, both with regards to the normal phenotype as well as disease susceptibility. The original hypothesis was phrased by Barker among others [5, 6], and fairly recently refashioned as the Developmental Origins of Health and Disease (DOHaD; [7–9]). It states that disease susceptibility originates from environmental exposures that programs cells, tissues, organs and systems of the body during critical periods in development. The mechanisms by which these programming events occur have

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not been entirely identified. However, epigenetic events have been shown to be responsible, at least in part, and alterations in epigenetic mechanisms may underlie an individual's propensity for health or disease over the span of a lifetime.

Epigenetic mechanisms control the way genetic information is maintained and used, and are defined as (heritable) alterations in gene expression levels and patterns not due to alterations within the primary DNA sequence (for further definition(s) see [10]). Epigenetic change can be environmental as well as developmental. Even though the DOHaD concerns both these concepts, it emphasizes the importance of the environmental aspects of epigenetic programming. It relates to nuclear environment and its interaction with permanent genetic features, such as sequence (e.g. genes) and sequence differences (e.g. single-nucleotide polymorphisms altering gene-function or affecting the binding of transcription factors that interact with the epigenome). Developmental epigenetic change occurs predominantly postpartum (dependent on species), and may lead to subsequent phenotypical alterations, adverse as well as beneficial, in the organism/individual.

One key epigenetic mechanism is DNA methylation, which involves addition of a methyl-group to the cytosine at CpG sites (cytosine followed by guanine linked together by a phosphate that separates all nucleosides in the DNA sequence), ultimately affecting expression of the gene in question. During embryogenesis, when the growing fetuses are particularly vulnerable to alterations *in utero*, DNA methylation is very dynamic. DNA is hypomethylated prior to implantation, with a progressive increase in methylation occurring following implantation leading to cellular differentiation and organogenesis [11, 12]. Cellular homeostasis and function in normal development is maintained by DNA methylation. In addition to regulating single genes, DNA methylation is important in broader contexts, such as in X-chromosome inactivation in females, regulation of chromatin structure, and genomic imprinting, to mention a few [13, 14]. Ziller *et al.* 2013 showed that DNA methylation covers approximately 70–90% of the CpG content of the mammalian genome, however, this is not homogenously distributed across the genome and varies by cell type [15]. Of note, in a study evaluating 42 whole-genome bisulphite sequencing data sets across 30 diverse human cell and tissue types, only about 20% of autosomal CpGs showed dynamic regulation within a normal developmental context. This occurred mostly at CpGs located distally to transcription start sites. However, significant co-localization of these dynamic CpGs with gene regulatory elements, such as enhancers and transcription-factor-binding sites was seen [15]. Other epigenetic mechanisms of interest, albeit less frequently examined in this context, include acetylations, histone modifications, and effects mediated by noncoding RNAs such as microRNAs (miRNA) and long noncoding RNAs.

Of these additional epigenetic mechanisms, miRNAs are of particular interest. miRNAs are small non-protein coding genes (~20–25 nucleotide (nt) duplex with 2 nt overhangs at the 3' ends) present in virtually all animals and plants that regulate gene expression post-transcriptionally. They function via complementary base-pairing with mRNA molecules, resulting in (predominantly) degradation of the target mRNAs. Importantly, only partial bas-pairing is needed in order to elicit translational repression, which has made it difficult to identify the targets for every miRNA. miRNAs are involved in regulation of many biological pathways and cellular functions, and are dynamically regulated during development. Notably, miRNAs were recently identified as regulators of maternal mRNA turnover during embryogenesis [16]. Emerging evidence reveals that epigenetic changes such as methylation and miRNA regulation are interrelated with each other in disease states such as different cancers [17, 18]. In rodent models of protein restriction during pregnancy, expression and function of miRNAs have been shown to be affected [19–21].

Genomic imprinting is a way by which genes are expressed in a parent-of-origin specific manner by methylation of the unexpressed allele [22–24]. Persistent shifts in DNA methylation at these imprinted genes may be an important mechanism by which the effects of poor fetal nutritional status are inherited. However, this is a question that is controversial and currently debated in scientific literature (for a discussion of transgenerational epigenetic inheritance see [10]). Transfer of epigenetic traits over one generation, from mother (F0) to fetus (F1), is well established. With regards to nutritional status, this has primarily been demonstrated for undernutrition, i.e. starvation, but evidence are also emerging for obesity [25].

The high caloric intake and imbalanced nutritional content that is part of the "westernized diet" may lead to adverse health effects in individuals. An association between maternal obesity and adverse health events in offspring has been demonstrated for diseases including cardiovascular disease, metabolic disorders, as well as behavioral health problems. Maternal high pre-pregnancy body mass index (BMI) as well as an increased rate of weight gain during pregnancy have been implicated in attention deficit-hyperactivity disorder (ADHD) and autism spectrum disorders [26–28]. The parental contribution to developmental programming by exposure to different nutritional environments has been mostly confined to evaluation of maternal contributions. However, some evidence from animal studies implicate an involvement of paternal pre-conceptual nutritional status on metabolic events in the offspring [25, 29].

The most famous study on maternal undernutrition was conducted on survivors of the Dutch Winter Hunger famine of 1944–1945. Here, a number of papers linked DNA methylation with perinatal malnutrition [30–32], which clearly established that maternal malnutrition is adverse to fetal development, and is a stressor affecting the adult individual. Maternal malnutrition during pregnancy, fetal growth and accompanied complications are most sensitive during peri-implantation and during the following rapid placental growth [33, 34]; both time-periods coinciding with DNA methylation dynamics. Maternal malnutrition leads to effects on multiple phenotypes and disease states in the offspring, including altered adipose tissue composition [35–37], thyroid [38] and hepatic function [38–40], as well as the well-examined increased risk for metabolic syndrome [41, 42] and cardiovascular disease [43, 44]. Additionally, maternal malnutrition may lead to an increase in fetal glucocorticoid exposure (see below) [45]. This is considered a primary mechanism by which maternal nutritional manipulation may result in long term endocrine adaptations in the fetus, and it has been shown to affect adulthood hypothalamic-pituitary-adrenal axis function [46–49]. However, opposing views do exist [50].

While the current review focuses on maternal contribution to developmental programming, it should be noted that increasing evidence exist of a paternal contribution as well. Paternal care of offspring is only present in 5–10% of mammals [51]. However, paternal effects on offspring occurs also in species with limited or no contact between father and offspring. A shown transgenerational inheritance, as well as presence of the phenomenon in isogenic species, hint to a role of epigenetic mechanisms in the paternal contribution to offspring phenotype [52–54]. In rodents, males with pre-conceptual dietary manipulations or exposure to toxins induce phenotypical traits

in offspring, also in subsequent generations (F2-F3) [55–58]. Although, studies exist contradicting the transgenerational inheritance [59]. In humans, a study showed that the epigenome of sperm from lean and obese men displayed marked differences in non-coding RNA expression and DNA methylation [60]. However, the extent to which these gametic epigenetic changes influence the metabolic profile of their offspring remains to be determined.

Offspring psychopathological phenotype is modulated by perinatal environment

Early environment affects brain development

In addition to the above mentioned effects on predominantly metabolic events and risk for metabolic disorders in adulthood, maternal nutritional status also modulates offspring affective states and mental health [26–28, 61, 62]. Brain development has been shown to be negatively affected by external adverse events during perinatal sensitive periods. These events impact both maturation of brain function and structure [63–66]. Physiological evidence for structural abnormalities include alterations in both gray and white matter, as well as possible loss of mass indicated by enlarged ventricular spaces, following perinatal adversities In particular, perinatal exposure to adverse events leads to structural abnormalities in regions that are involved in regulation of emotionality. These regions include, but are not limited to, limbic structures such as the amygdala and hippocampus, as well as hypothalamic nuclei [63, 67, 68]. Additionally, perinatal stress exposure either via administration of glucocorticoids or behavioral stress affects neurotransmitter systems in the brain [69–72]. These disruptions may underlie effects of perinatal stress on adulthood mental health and psychopathology.

Regulation of affect, i.e. the experience of emotion, is maintained by complex biological events and results from Gene x Environment interactions. We know today that for these complex states, significant contributions of both “nature” and “nurture” are combined into the final state of each individual. For affective phenotypes, it has been determined that seemingly similar phenotypic traits, may have varying underlying contributions from genetics, as well as environmental influences [73–78]. Most likely, significant overlap between the two is modulated by epigenetic mechanisms [76, 79].

Stress during pregnancy affects offspring stress-sensitivity over generations

The fetus is to a large extent protected by the placental barrier, which limits passage of neurotransmitters and hormones from mother to fetus across the placenta. However, stress during pregnancy has been shown to modulate neuroendocrine function in both mother and fetus.

For glucocorticoids (cortisol in humans, corticosterone in rodents), the enzyme 11beta-hydroxysteroid dehydrogenase type 2 (11HSDB2) catalyzes a reaction leading to inactivation of the respective stress-hormones, and it is highly expressed in the placenta [80–82]. Glucocorticoids are involved in modulation of stress-effects on fetal brain development and, thereby, affect fetal programming events [83, 84–87]. Increased exposure to stress-hormones during pregnancy leads to altered hypothalamic-pituitary-adrenal functioning postnatally [88]. The hippocampus-HPA axis feedback loop involving the glucocorticoid receptor (GR) is also altered by maternal stress. Here, altered expression of the hippocampal GR indicates effects on offspring emotional reactivity, thereby linking stress-exposure with effects within the central nervous system (CNS) [45, 89–91]. In animal and human populations, HPA-axis dysfunction is known to be related to mental health [92–96], and the disruptive effects of perinatal stress on glucocorticoid function, both centrally and in the periphery, may pre-dispose offspring to an increased risk of experiencing mental health problems in adulthood. In fact, this has been demonstrated in both animal and human studies [88, 97–103]. Specifically, perinatally stressed rats show a high degree of emotionality in animal behavioral models, such as decreased open arm exploration in the elevated plus-maze, more defensive freezing, decreased exploratory behavior accompanied by increased defecation, and impaired cognitive function [104–107].

In addition to endocrine stress, exposure to environmental endocrine-disrupting chemicals during pregnancy is adverse to offspring health and reproduction. It may cause general health as well as reproductive defects that may be inherited transgenerationally via epigenetic mechanisms [108–111]. A pronounced behavioral trait in offspring from exposed dams, or from a chemical exposed lineage, is differential stress sensitivity persisting over generations, with exposed offspring being more anxious in animal models such as the open field [111]. It has been reported that perinatal stress is associated with a significant decrease in 11HSDB2 mRNA, increased mRNA levels of the DNA methyltransferase DNMT3a, and increased DNA methylation at specific CpG sites within the 11HSDB2 gene promoter in the placenta [112].

In summary, stressful experiences such as those leading to elevated endocrine response, or exposure to toxins, lead to offspring with health problems including reproductive issues, affective health, and stress-responsiveness. In addition, this effect may persist across generations.

Malnutrition mimics stress during pregnancy

Malnutrition has been shown to have stress-like effects on fetal development. Malnutrition involves endocrine mechanisms [33, 47, 113], and has been shown to lead to an increased risk for development of metabolic syndrome in the offspring [41, 114, 115].

In order to understand the underlying mechanisms of epigenetic programming affecting the adult offspring phenotype induced by maternal malnutrition, animal models have frequently been used [116–118]. In rodents, models using diets to induce effects on offspring usually employ either a high-fat diet to model a “westernized diet” and diet-induced obesity, or a low protein diet/calorie restriction to mirror components of general malnutrition. Dietary modulations can be done in combination with exposure to stressors. Swim stress and cold stress are among those often used. The combination of a high-fat diet following perinatal exposure to a stressor is more likely to produce a state in the offspring similar to the metabolic syndrome seen in humans than either exposure alone [117]. High fat diet induces alterations in insulin and glucose metabolism, energy balance, cardiovascular function, and adiposity in offspring. A low protein diet predominantly results in subsequent sensitivity to development of metabolic syndrome, effects on immune-related functions [46, 47, 119], as well as energy balance, cardiovascular function, and diabetes risk [43, 120].

Maternal low protein diet has been shown to lead to reduced fetal growth and decreased weight at birth. Additionally, low protein diet leads to increased fetal exposure to corticosterone via reduced elimination in the placenta, in mice as well as rats [47]. However, the processes by which fetal growth is repressed by maternal malnutrition or glucocorticoids are distinct [45].

The effect of a low protein diet on maternal and fetal stress-hormone has also been shown in other species such as pigs [121]. Additionally, maternal malnutrition has been correlated with affective disorders in offspring [122–125].

Offspring effects of maternal malnutrition are due to epigenetic mechanisms

As for the mechanisms of action, regardless of the type of insult during pregnancy, there appears to be similar effects on fetal growth, neurodevelopment, and metabolism [126], indicating common pathways. Since all insults impact the HPA axis, which represents a well-studied link between adaptations in the CNS following environmental stress and peripheral metabolic responses [127–129], this is not surprising. However, while the events leading up to altered HPA-axis function were for a long time unknown, today there is ever increasing evidence towards an epigenetic explanation [88, 130]. However, while the HPA-axis is of central importance, other mechanisms are most likely of equal, if not greater, significance particular in relation to mental health. Using animal models as well as human studies, it has been shown that perinatal stress-exposure modulates epigenetic mechanisms on both a genome-wide scale as well as for individual genes [12, 19, 25, 31, 98, 131–140]. Many of the identified genes with altered DNA methylation and expression following perinatal malnutrition are neuropeptides.

Neuropeptides CRH and NPY in regulation of energy homeostasis and affective-like behaviors

Within the CNS, there exist neuropeptide-systems regulating, among other things, energy homeostasis and affective behaviors through partially overlapping systems. Brain regions of importance in the regulation of these behaviors include the hypothalamus, amygdala, and related structures such as the bed nucleus of the stria terminalis and the ventral tegmental area and nucleus accumbens (see for example [141–143]). Many neuropeptides are involved in regulating opposite phenotypes, including orexigenic/anxiolytic agents such as neuropeptide Y (NPY) and anorectic/anxiogenic agents such as corticotropin-releasing hormone (CRH). Neuropeptides exert their functions via g-protein coupled receptors, often with multiple receptor subtypes regulating different aspects of a neuropeptide's functions. Commonly, neuropeptides are not highly constitutively expressed. Instead their expression and, thus, function, is triggered by external or internal stimuli, leading to a response that is slower and more long-lasting than that of classical neurotransmitters. Often different neuropeptide system have opposite functions within a brain area generating a tightly regulated unit which can initiate and stop behavioral, endocrine, and other responses as needed (for example see [144, 145]). Additionally, many neuropeptides have both peripheral and central functions. Here, we will focus on the previously mentioned NPY and CRH, and put them in context of perinatal nutrition and epigenetic regulation.

Neuropeptide Y

NPY, a 36 amino acid peptide highly expressed in limbic regions of the brain, including the hypothalamus and amygdala, as well as the hippocampus [146], was isolated in 1982 at Karolinska Institutet, Stockholm, Sweden by Tatemoto and co-workers [147]. The peptide exerts its effects via actions at six different receptor subtypes, of which the Y1, Y2 and the Y5 subtypes are of greatest importance in humans and rodents [148]. With regards to behavioral regulation, NPY is, as mentioned, orexigenic, primarily stimulating intake of carbohydrates, via actions in the hypothalamus. Within the hypothalamus, the peptide is synthesized predominantly in the arcuate nucleus (ARC), and exerts its effects in other subnuclei including the paraventricular (PVN), dorsomedial (DMN) and ventromedial (VMN) nuclei, and the perifornical area [149, 150]. The activity within the hypothalamus is further modulated by the hindbrain and limbic structures [151]. NPY activity decreases latency to eat, increases motivation to eat and delays satiety by augmenting meal size predominantly via actions at the Y1 and Y5 receptors [152, 153]. With regards to regulation of affective behaviors such as anxiety- and depression-related behaviors, NPY is anxiolytic within the amygdaloid complex through actions at the Y1- receptor subtype, a region regulating fear-related behavior [154–158].

Corticotropin-releasing hormone

CRH [159], a 41 amino acid peptide also highly expressed within the CNS in regions involved in behavioral and endocrine responses to stress such as the hypothalamus and the amygdala, exerts its actions via two main receptor subtypes, the CRHR1 and the CRHR2 [160–162]. Within the hypothalamus, CRH is produced in the paraventricular nucleus, and is catabolic, as well as anorectic [163–165]. Additionally, CRH in the hypothalamus regulates the HPA-axis and functions as a "relay" between central and peripheral actions [166]. The CRH neurons in the PVN are of importance in the neurobiology of depression, since a subset of patients with depression display a hyperactive HPA-axis, which is driven by these neurons. Within the PVN NPY release triggers subsequent release of CRH, and there is a negative feedback loop of CRH on NPY peptide synthesis and release [163]. In the amygdala CRH is anxiogenic, primarily via actions on the CRHR1 receptor subtype [162], and also here there is a "yin-yang" relationship with NPY [167]. It should be noted that while there is some communication between the amygdala and hypothalamus, regulation of food intake and HPA-axis activity within and beyond the hypothalamus is differentially regulated compared to anxiety- and depression- related behaviors, which have their neurobiological basis in the amygdala [168, 169].

The differential contributions of NPY and CRH indicate that they are both of interest in the metabolic syndrome which includes HPA-axis dysfunction and altered energy balance, as well as in affective disorders.

Epigenetic regulation of neuropeptide function—CRH

Increasing evidence point to epigenetic regulation of both the NPY- and CRH –system components, including the peptides themselves as well as their receptors. For CRH a number of studies exist evaluating post-natal stress and epigenetic regulation of CRH expression. The postnatal period in rodents (days 1-appr. 20) is approximately equivalent to the third trimester in human pregnancies and is extremely critical for the development of normal brain function. A model using repeated postnatal maternal separation is one of the most potent stressors to which neonates can be exposed, and it has been shown to influence the adult phenotype [170]. Postnatal maternal separation does lead to impaired stress coping and memory function, as well as a lasting hyperactivity within the HPA- axis. This is accompanied by increased CRH mRNA and protein expression in the hippocampal CA1 of adult rats.

With regards to epigenetic mechanisms, both increased histone H3 acetylation and decreased DNA methylation has been detected in the CRH promoter region following maternal separation in rats. Decreased occupancy of Methyl CpG binding protein 2 (MeCP2), a transcriptional factor suppressing the expression of target genes, was shown to be involved in the mechanism underlying these alterations. MeCP2 generally binds methylated CpG sites in the promoter region and recruits several other transcriptional factors to modify histone acetylation and DNA methylation. Occupancy of HDAC2, a transcriptional repressor, was also decreased leading to increased histone H3 acetylation in the CRH promoter region within the hippocampal CA1 of maternally separated rats. Finally, also DNA methyltransferase 1, which may interact with MeCP2 to maintain the 5′-cytosine methylation in the promoter region of target genes, was reduced leading to decreased methylation of 5′-cytosine in the region. Taking together these results imply that the histone hyperacetylation and DNA hypomethylation mediated by MeCP2 and associated transcriptional factors might underlie the upregulation of hippocampal CRH- expression, and function in the rats with postnatal maternal separation. This may in turn affect the hippocampus-HPA feedback loop and thereby lead to negative behavioral consequences such as a hyperactive HPA-axis or even depression [94, 96, 170, 171]. Notably, the hippocampus is also of importance in fear related memories and has strong bidirectional connections with the amygdala.

Within the amygdala, the CRHR1 receptor has been implicated as a "plasticity gene" for anxiety-related behavior in mice. It was shown to be regulated bidirectionally, in a manner dependent on the type of environmental stimuli (anxiolytic or anxiogenic). Within the amygdala, a CpG1 site located at 1348 bp upstream of the CRHR1 promoter was found to be differentially methylated in mice bred for high (HAB) or low (LAB) anxiety phenotype, and was also determined to be adjacent to a binding site for a transcription factor, Yin-Yang 1 [172]. Additionally, it was shown that early life stress and 5-HTT genotype interact to affect DNA methylation of the CRH gene promoter in the central nucleus of the amygdala of adult male rats [173]. Taken together this data implicate epigenetic mechanisms in regulation of CRH and CRHR1 gene expression and function within the hippocampus and amygdala.

Hyperactivity of the HPA-axis has been shown in both female and male rat offspring following exposure to maternal separation between postnatal days 2 and 13. Plasma corticosterone levels and PVN CRH heterogeneous nuclear (hn)RNA (a precursor to mRNA) responses to acute restraint stress, were higher in stressed rats, and DNA methylation analysis of the CRH promoter revealed a significantly lower percentage of methylation in two CpG sites; one preceding and another inside the cyclic AMP-response element (CRE) which is located 230 bp upstream of the CRH [174]. Epigenetic mechanisms may thus partake in regulation of CRH expression and function within the hypothalamus, indicating a role in regulation of the HPA-axis.

The above mentioned studies were performed postnatally in rodents at a time-period corresponding to the third trimester in human pregnancy, making the studies valuable when evaluating perinatal effects of stress on offspring adult phenotype. With regards to prenatal stress in rodents, it was shown that exposing pregnant rat dams to restraint stress lead to a hyperactive HPA-axis in the offspring, as well as demethylation of the CRH- promoter in the hypothalamus [175].

With regards to miRNAs and CRH, it has been shown that deletion of Dicer, an enzyme cleaving pre-miRNAs into miRNAs, in mice leads to decreased expression of CRH as well as CRHR1 mRNA within the hypothalamus [176]. Otherwise, data on miRNA involvement in regulation of CRH expression is very limited.

CRH, both within the hypothalamus in relation to HPA-axis activity, as well as within extra-hypothalamic sites such as the amygdala and hippocampus, is subject to epigenetic mechanisms regulating its expression and function in response to stressors. This is also true for the CRHR1- subtype of its receptors.

Epigenetic regulation of neuropeptide function—NPY

Epigenetic mechanisms have so far only to a limited extent been shown to be involved in stress- and nutritional regulation of NPY expression and/or function. A low protein diet, previously shown to influence development of peripheral events indicative of metabolic syndrome [117] has been used to evaluate epigenetic mechanisms in regulation of gene expression of NPY and its receptors. In mice, maternal low protein diet exacerbated anxiety- and depression like behavior in male offspring in adulthood, while female offspring appeared to be "protected" against this phenotype. In association with this, within the amygdala maternal low protein diet lead to increased DNA-methylation in the first intron of the Npy1r gene in females but not in males (Natt *et al*. Manuscript in preparation), which might have affected stress resilience in female offspring.

Perinatal malnutrition has stress-like effects on offspring in animal models [121, 177–180] and has been shown to alter DNA methylation of CpG dinucleotides in the proximal promoter region of the NPY gene within the hypothalamus at 16- and 100-days of age, compared to control rats. Additionally, rearing of newborn rats on a high-carbohydrate diet shown to induce hyperinsulinemia, was shown to increase acetylation of lysine 9 in histone 3 (H3K9) for the NPY gene, without changes in histone methylation (H3K9). These findings were consistent with the changes in the expression levels of NPY. While not being a proper perinatal manipulation *per se*, these results suggest that epigenetic mechanisms regulate NPY levels in response to nutritional stress, at least within the hypothalamus.

In relation to affective disorders, a SNP in the rat NPY-gene promoter (C/T; rs105431668) affects *in vitro* transcription and DNA-protein interactions. In a rat model of depression, the Flinders sensitive line and its counterpart, the Flinders resistant line, the presence of the C-allele enables binding of a transcription factor (CREB2) and a histone acetyltransferase (Ep300). It was determined that the C-allele is only present in the Flinders resistant rat line, and that its presence correlates with increased hippocampal levels of NPY mRNA and H3K18 acetylation; a gene-activating histone modification maintained by Ep300 [181]. This finding illustrates a direct epigenetic mechanism for regulation of NPY expression and function. The presence of the SNP within a selectively bred line of rats may limit its usefulness; however, this needs to be evaluated by determining the frequency of the C-allele in different outbred populations. At the very least, the finding opens up an interesting avenue of exploration for genetic/epigenetic interactions in affective disorders. Furthermore, this suggests that different populations due to their genetics may be differentially susceptible to exposure to stressful, adverse events both during development, as well as in adulthood.

With regards to miRNAs and NPY, the same study examining CRH within hypothalamus indicated that deletion of Dicer, an enzyme cleaving pre-miRNAs into miRNAs, in mice leads to decreased expression of NPY mRNA within the hypothalamus

[176]. However, the authors indicate that this may be a compensatory mechanism due to the genetic modification, and not a direct cause-effect relationship. Thus, miRNA involvement in NPY gene expression remains to be elucidated.

In summary, the epigenetic mechanisms for regulation of NPY expression and function, are currently only described in a very limited literature. Due to the involvement of CRH in the regulation and maintenance of the HPA-axis, much more information is available here with regards epigenetic regulation of gene expression.

Conclusion

Complex events regulate and maintain behaviors, and modulate disease risk throughout an individual's lifetime, starting already at conception. Early indications of perinatal events influencing the adult phenotype were first presented in relation to cardiovascular risk following malnutrition during pregnancy. This has since been expanded to include many disease-states related to energy metabolism, but also in relation to stress-sensitivity as well as psychiatric (affective) disorders. Maternal stress, physiological, psychological, as well as nutritional, affects HPA-axis function in adult offspring. This is often seen as a hyperactive response to stressful stimuli, and an increased prevalence of affective disorders.

Epigenetic mechanisms have been implicated in regulation of these phenotypic effects, wherein particular alterations in DNA methylation have been well studied. Of great importance the last ten years, have been to elucidate the epigenetics of stress and how that relates to glucocorticoid regulatory enzymes, receptors, and to HPA-axis function.

Given the actions and interactions of neuropeptides NPY and CRH, and their roles in regulation of both energy homeostasis as well as affective behavior, we here examined evidence for epigenetic mechanisms to be involved in regulation of their expression and function. Epigenetic mechanisms, in particular methylation, are involved in regulation of these systems, thereby making them putative mechanistic targets for the effects of maternal stress on offspring behavior. However, more work is needed in order to further elucidate mechanism(s) and function of these two neuropeptide systems in relation to maternal stress effects in adult offspring.

Conflict of interest statement. None declared.

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