European Journal of Pharmacology 660 (2011) 194–201

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Review

Epigenetic changes in the hypothalamic pro-opiomelanocortin gene: A mechanism linking maternal undernutrition to obesity in the offspring?

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ARTICLE INFO

Article history:
Received 9 August 2010
Received in revised form 6 October 2010
Accepted 29 October 2010
Available online 3 January 2011

Keywords:
POMC
Hypothalamus
Glucocorticoid receptor
Maternal undernutrition
Programming
Epigenetics

ABSTRACT

Maternal undernutrition is associated with programming of obesity in offspring. While previous evidence has linked programming to the hypothalamic, pituitary, and adrenal (HPA) axis it could also affect the hypothalamic neuropeptides which regulate food intake and energy balance. Alpha melanocyte stimulating hormone (αMSH), a key regulator of these neuronal pathways, is derived from pro-opiomelanocortin (POMC) which is therefore a prime target for the programming of obesity. Several models of maternal undernutrition have identified changes in POMC in hypothalami from foetuses or offspring at various ages. These models have also shown that the offspring go on to develop obesity and/or glucose intolerance. It is our hypothesis that programming leads to epigenetic changes in hypothalamic neuropeptide genes. Therefore when there is subsequent increased food availability, the epigenetic changes could cause dysfunctional transcriptional regulation of energy balance. We present evidence of epigenetic changes in the POMC gene promoter in foetal hypothalami after peri-conceptional undernutrition. In this model there are also epigenetic changes in the hypothalamic glucocorticoid receptor with consequent up-regulation of the receptor which could lead to alterations in the regulation of POMC and neuropeptide Y (NPY) in the hypothalamus. Thus maternal undernutrition could cause epigenetic changes in the POMC and glucocorticoid receptor genes, in the foetal hypothalamus, which may predispose the offspring to altered regulation of food intake, energy expenditure and glucose homeostasis, later in life.

Contents

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0014-2999/$ – see front matter.
doi:10.1016/j.ejphar.2010.10.111

1. Introduction

Hypothalamic POMC is implicated in the development of both obesity and diabetes, each of which has a major impact on human health. Indeed, metabolic syndrome, which includes obesity, cardiovascular disease and diabetes, affects up to 25% of the population in the USA (Ford et al., 2002). Although there is increasing evidence for candidate genes involved in the development of obesity there is still a lack of clarity of how this occurs in the majority of cases. One area of interest is the effect of maternal nutrition on obesity in the offspring. This association was identified in survivors of the Dutch famine of 1944–1945 where there was a correlation between maternal undernutrition and subsequent obesity in adult offspring (Ravelli et al., 1976). This led to the concept being termed maternal programming (Seckl, 2004).

Programming of the foetus might occur to prepare the offspring for an adverse postnatal environment (Kapoor, 2006). However these changes can be associated with offspring having lower birth weight, increased propensity to develop an obese phenotype in later life and increased susceptibility to cardiovascular disease and diabetes (Martin-Gronert and Ozanne, 2005; Rhodes et al., 2009).

There are many factors that might influence the outcome of developmental programming. These include the timing of the insult, the number of foetuses and the sex of the offspring. Furthermore the type of insult is also important, which along with stress (Seckl and Holmes, 2007) includes nutritional effects that could be mediated by the hypothalamus (Bertram and Hanson, 2002).

2. Programming of food intake and energy balance

2.1. Neuropeptide regulation of food intake

Energy homeostasis is tightly controlled in the hypothalamus despite fluctuations in energy consumption and expenditure. Therefore the foetal hypothalamic appetite regulatory network is a prime candidate for maternal programming. Hypothalamic regulation of food intake and energy balance in the arcuate nucleus (Fig. 1) depends on a complex array of neuropeptides but most is known about the anorexigenic pro-opiomelanocortin (POMC) and cocaine–amphetamine regulated transcript expressing neurons and the orexigenic neuropeptide Y and agouti related peptide expressing neurons (Challis and Yeo, 2002). The activation of POMC neurons results in the cleavage of the prohormone, POMC, to αMSH and the release of αMSH in the paraventricular nucleus (Pritchard et al., 2003, 2002; Pritchard and White, 2007).

In situations of positive energy balance, leptin released from adipocytes, stimulates the anorexigenic pathway and inhibits the orexigenic pathway (Plum et al., 2006; Pritchard and White, 2007). This results in a reduction in food intake. Conversely in situations of negative energy balance, the orexigenic pathway is activated. This pathway acts to increase food intake in part by agouti related peptide, preventing αMSH from binding to the melanocortin MC_3 and MC_4 receptors in the paraventricular nucleus (Cripps, 2005; Pritchard et al., 2004) (Fig. 1). It has been shown that components of adult energy balance regulation are present in the hypothalamus as early as mid-gestation in humans (Adam et al., 2008). POMC is expressed extensively in immature hypothalamic neurons in rat foetuses but subsequently half of this population of neurons adopts a non-POMC fate, including development into neuropeptide Y neurons. Up to a quarter of neuropeptide Y neurons have an immature POMC neuron progenitor (Padilla et al., 2010).

2.2. Regulation of POMC expression in the hypothalamus

Feeding and hormonal signals regulate expression of the POMC gene in the hypothalamus and therefore the associated secretion of

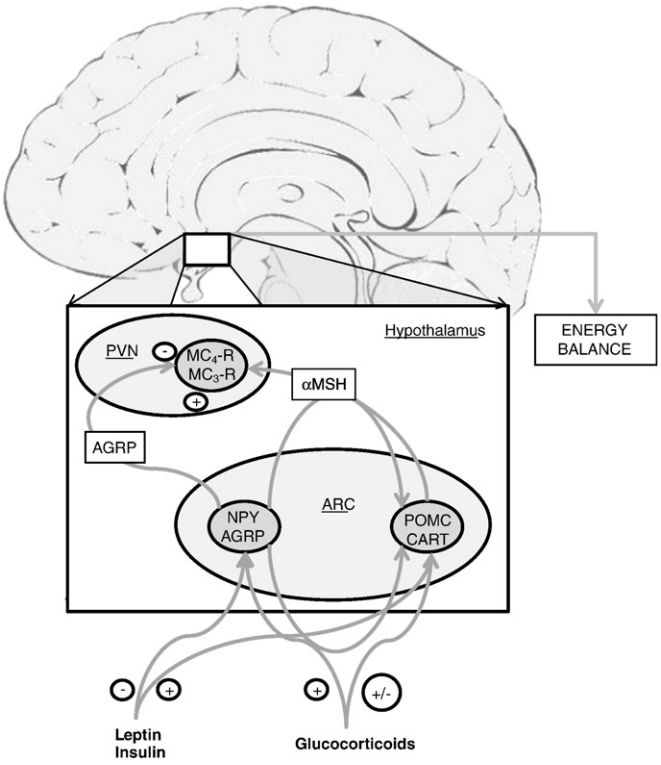

Fig. 1. The hypothalamic control of energy balance. ARC = arcuate nucleus, PVN = paraventricular nucleus; AGRP = agouti related peptide, POMC = pro-opiomelanocortin, CART = cocaine-amphetamine regulated transcript, NPY = neuropeptide Y, MC_4-R = melanocortin receptor 4, MC_3-R = melanocortin receptor 3.

POMC-derived peptides (Cone, 2005; Pritchard et al., 2003, 2002; Pritchard and White, 2007). Hypothalamic POMC neuronal excitability, hypothalamic POMC mRNA and circulating αMSH are decreased in rodent models of leptin or leptin-receptor deficiency (Forbes et al., 2001; Korner et al., 1999; Korner et al., 2001; Mizuno et al., 1998; Thornton et al., 1997; Turner et al., 2006) and this can be reversed by leptin administration (Cowley et al., 2001; Forbes et al., 2001; Korner et al., 2001; Schwartz et al., 1997; Thornton et al., 1997).

It is well known that in the pituitary, POMC transcription is inhibited by glucocorticoids. Hypothalamic expression of POMC can also be regulated by glucocorticoids, but there are differing observations on how this occurs and these are discussed further in Section 4.3. Some POMC neurons also possess receptors for insulin and co-administration of the melanocortin MC_4 receptor antagonist, SHU9119, prevents the anorectic effects of insulin (Benoit et al., 2002). Reductions in POMC mRNA levels were observed in conjunction with decreased plasma insulin concentrations in adrenalectomised versus sham-operated rats demonstrating the regulatory effect of glucocorticoids on this process (Savontaus et al., 2002) (Fig. 1). Moreover, decreases in hypothalamic POMC mRNA caused by fasting were prevented by intracerebroventricular (i.c.v) injection of insulin (Benoit et al., 2002). POMC neurons are also regulated by glucose and they express components of the glucose-sensing ATP-sensitive potassium channels and respond to a reduction in extracellular glucose concentration by decreasing neuronal firing (Gyte et al., 2007; Ibrahim et al., 2003). This suggests a key role for POMC in the central melanocortin regulation of glucose homeostasis (Obici et al., 2001).

2.3. The role of POMC in food intake and energy balance

The importance of POMC in food intake and energy balance is exemplified by mutations in the POMC gene resulting in an obese phenotype which is primarily due to loss of ligands for melanocortin

MC_3 and MC_4 receptors in the brain (Krude et al., 2003). Patients with mutations in POMC demonstrate early-onset severe obesity and distinctive red hair (Krude et al., 1998, 2003; Krude and Gruters, 2000). Targeted disruption of the POMC gene results in hyperphagia and lower oxygen consumption in mice, causing increased fat mass and obesity (Challis et al., 2004; Yaswen et al., 1999). Mutations in the peptide processing enzyme prohormone convertase 1 render patients unable to process POMC to functional peptides and are associated with severe early-onset obesity (Jackson et al., 1997). Another mutation has been identified in the human POMC gene that can confer an inherited susceptibility to obesity through the production of an aberrant fusion of beta MSH to beta endorphin that has the capacity to interfere with central melanocortin signalling (Challis et al., 2002). Food deprivation results in significant decreases in hypothalamic POMC mRNA levels and reduced release of POMC-derived peptides from hypothalamic slices (Breen et al., 2005; Korner et al., 2001; Swart et al., 2002). Intracerebroventricular injection of POMC-derived peptides inhibits food intake in rats, even after a 24-hour fast (Poggioli et al., 1986; Vergoni et al., 1990).

3. Adverse maternal nutrition programs hypothalamic feeding centres

Given that the epidemiological analyses of large cohorts of patients have suggested that maternal undernutrition impacts on obesity in the offspring (Rhodes et al., 2009), several groups have investigated how models of undernutrition during gestation affect food intake and energy expenditure.

3.1. Programming of hypothalamic neuropeptides with maternal undernutrition in the rat

Prenatal undernutrition together with a postnatal high fat diet produces obese characteristics, but this did not occur with maternal undernutrition alone (Ikenasio-Thorpe et al., 2007). This study suggests that the combination of changes in the prenatal and postnatal environment, leads to dysregulation of the hypothalamic appetite regulatory network, which might contribute to an obese phenotype.

The same model of maternal undernutrition has been used to examine foetal hypothalamic anorexogenic pathways. Changes in hypothalamic gene expression and neuronal activation were identified which could lead to the development of obesity in later life. These changes included a reduction in postnatal plasma leptin levels, a decrease in POMC mRNA levels and a decrease in the number of POMC nerve fibre projections from the arcuate nucleus to the paraventricular nucleus (Delahaye et al., 2008). Using a more severe model of undernutrition (70% reduction in maternal rat food intake from day 1 to day 21 of gestation), there were no differences in POMC and neuropeptide Y mRNA expression between control and undernourished adult rats. However, following fasting there was an increase in neuropeptide Y mRNA and in the activity of POMC neurons in the arcuate nucleus (Breton et al., 2009).

In rats, POMC is expressed as early as embryonic day 12 (E12) (Terroni et al., 2005). E12 is correlated with massive cell proliferation, leading to hypothalamic differentiation (Markakis, 2002) and POMC is highly expressed in the immature neurons at this stage (Padilla et al., 2010). Furthermore, maternal protein restriction in rats from conception to E12 results in changes in the foetal hypothalamic regulatory network, with increased expression of agouti related peptide, neuropeptide Y, POMC and the leptin receptor (Ob-Rb). These changes may indicate that the appetite regulatory pathway is active in the foetus (Terroni et al., 2005) although these pathways do not fully mature in the rat until after birth (Symonds and Budge, 2009). As a result the foetal developmental events observed in the rat might be significantly different to those observed in humans.

3.2. Effects of maternal undernutrition on foetal programming in sheep

Studying programming in sheep has the advantage that they have greater developmental similarity to humans because they give birth to mature young, have a high frequency of singleton pregnancies and have a longer gestational period. Our studies have identified programming of foetal hypothalamic regulatory pathways and while no change was observed in foetal hypothalamic POMC expression, there was increased glucocorticoid receptor expression which was associated with maternal undernutrition (Stevens et al., 2010). Adult sheep which have undergone a similar phase of maternal undernutrition were found to have increased body weight and glucose intolerance (Rumball et al., 2009; Todd et al., 2009).

In our study maternal ewes were undernourished to achieve a 10–15% reduction in body weight around conception (i.e. for 60 days before to 30 days after conception) (Rumball et al., 2009; Stevens et al., 2010). A more severe model of maternal undernutrition in the sheep used a 50% reduction in food intake between 30 and 80 days of gestation. It was found that there was no effect on birth weight or growth of the offspring, and while there was no change in hypothalamic POMC expression in the foetus, after one week of age hypothalamic POMC levels increased (Sebert et al., 2009).

3.3. Effects of maternal overnutrition on programming

It has been proposed that maternal obesity could exert a stronger impact in offspring than postnatal overnutrition and with the increase in obesity in the population it is likely to be a significant problem in the future. In a study where male rats born to obese dams were heavier than controls, they had a rather surprising change in neuropeptides with increased hypothalamic POMC mRNA expression and reduced neuropeptide Y (Chen et al., 2008). Maternal hyperglycemia was also associated with upregulated hypothalamic POMC in the foetuses of sheep at mid-gestation (Muhlhausler et al., 2005).

4. Epigenetic effects on hypothalamic POMC pathways regulating food intake and energy balance

Epigenetic modifications of key genes have been proposed as a potential underlying mechanism for foetal programming (Newell-Price, 2003). Epigenetic modifications regulate chromatin assembly, chromosome separation, the replication and repair of DNA and gene expression. All these processes are necessary for gametogenesis and the development of the foetus (Delage and Dashwood, 2008).

The state of the chromatin structure, with its complex of histone proteins surrounded by DNA forming “bead-like” nucleosomes, determines whether it is transcriptionally activated or inactivated. Open chromatin is associated with activation and the closed chromatin is inactive (McGowan et al., 2008). Thus, epigenetic modifications act to transcriptionally silence or activate genes by altering the chromatin structure without affecting the DNA sequence. Two methods by which this can occur are DNA methylation and modification of histone tails (acetylation and methylation) (Ho and Tang, 2007) (Fig. 2).

DNA methylation is associated with gene silencing, preventing the DNA from undergoing transcription (Fig. 2). Its effects are thought to be permanent and it occurs at cytosine residues throughout the genome. Particularly dense regions of DNA methylation associated with gene activity are termed CpG islands, which can be 200–2000 base pairs in length. The islands usually extend over transcriptional start sites (Ho and Tang, 2007). Patterns of DNA methylation are set during foetal development. Initially non-specific demethylation occurs and then *de novo* methylation, followed by specific demethylation (Newell-Price, 2003). Thus, it is possible to speculate that the patterns of methylation are potentially vulnerable to changes in the surrounding foetal environment.

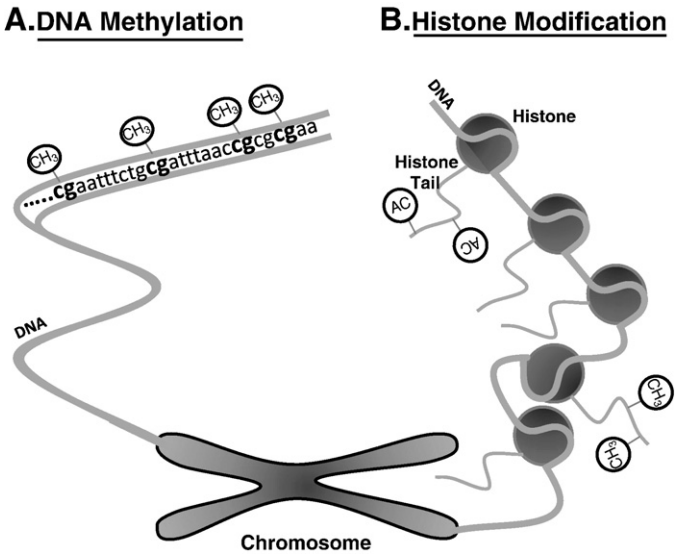

Fig. 2. Epigenetic modifications of chromatin. A) Methylation of cytosine bases in CpG islands leads to transcriptional repression. B) The modification of histone "tails" alters transcriptional activity, usually acetylation is associated with transcriptional activation and the methylation is associated with transcriptional repression.

In contrast to DNA methylation, histone tail acetylation (and some changes in histone methylation) are associated with transcriptional activation of a gene. Conversely histone tails can be modified in such a way as to cause transcriptional repression usually via deacetylation or methylation and this is correlated with inactive chromatin (Graff and Mansuy, 2008).

Human POMC is known to have 2 CpG islands, one over exon 1 and the associated promoter region and one downstream over exon 3 (Newell-Price, 2003). It is possible to speculate that in the obese phenotype there would be alterations in the POMC methylation status, which would lead to decreases in POMC expression. As a result there could be an increase in food intake. Changes in POMC gene promoter methylation states have been seen in tissues and cell lines from human cancer and are associated with changes in POMC expression (Mizoguchi et al., 2007; Newell-Price et al., 2001; Ye et al., 2005). POMC transcriptional enhancer regions associated with hypothalamic expression have been identified 10–12 kb upstream of the start site of the POMC gene (de Souza et al., 2005). While the POMC CpG islands do not overlap the regions associated with hypothalamic expression, we have defined further CpG rich regions 4 kb downstream of the hypothalamic region which bind RNA polymerase II and are associated with acetylated histone H3K9 (Stevens et al., 2010) implying that epigenetic changes may have a role in the modulation of hypothalamic POMC gene expression (Fig. 3).

4.1. Epigenetic changes in the foetus associated with maternal undernutrition

In rodents, several studies suggest that hypothalamic programming begins *in utero* but continues in early postnatal life during the suckling period, leading to long-lasting dysfunction in adulthood. These studies have shown that the hypothalamic control of appetite is a key target of perinatal developmental programming, possibly disturbing body weight set point (Coupe et al., 2010). There is also data to suggest that maternal undernutrition differentially affects the appetite regulatory system of offspring long-term via the response of POMC neurons to energy status and food intake (Breton et al., 2009).

It could be that maternal nutritional changes cause epigenetic programming in the foetus. One investigation involved feeding a low protein diet to pregnant rat dams. Following methylation analysis, specific POMC CpG sites were found to be less methylated in the protein-restricted offspring compared to controls (Coupe et al., 2010). This study suggests that maternal nutritional insults can induce epigenetic programming in foetal pathways.

Recently we have used a sheep model of maternal undernutrition and found epigenetic changes in foetal hypothalamic regulatory pathways (Stevens et al., 2010). A promoter region marker for the POMC gene was identified in the sheep using comparative homology and shown to be associated with histone H3K9 acetylation, implying that the gene was accessible (Fig. 4A). There was also hypomethylation of the POMC promoter marker in the hypothalami of foetuses exposed to maternal undernutrition (Fig. 4B). However transcription of the hypothalamic POMC gene was not increased (Fig. 4C and D). We therefore suggested that the hypothalamic POMC gene may be primed for transcriptional activity which was not yet measurable prior to parturition (Stevens et al., 2010).

4.2. Epigenetic changes in POMC after maternal overnutrition

Little is known about the association of epigenetic changes in the offspring with overfeeding in mothers. Neonatal overfeeding in rats was associated with hypomethylation of the hypothalamic POMC gene promoter at two sites involved with leptin and insulin modulation of POMC expression. However despite the alteration in epigenetic status there was no change in POMC expression between control and overfed rats (Plagemann et al., 2009). It could be that the change in expression may become manifest later in life, resulting in the maternally overfed group becoming more susceptible to obesity.

4.3. Modulation of hypothalamic POMC as a result of epigenetic changes in the glucocorticoid receptor

In our studies investigating epigenetic changes in the POMC promoter marker after maternal undernutrition, we also investigated epigenetic changes in the hypothalamic glucocorticoid receptor and found hypomethylation of this marker associated with a variety of periconceptional maternal undernutrition regimens (Fig. 5A); along with increased hypothalamic expression of the glucocorticoid receptor (Fig. 5B) within the arcuate nucleus of the foetal hypothalamus (Fig. 5C) (Stevens et al., 2010). Therefore we cannot discount the possibility that the prime candidate for epigenetic changes is the glucocorticoid receptor which mediates regulation of a number of hypothalamic neuropeptides including POMC and neuropeptide Y.

While there is a well-defined feedback mechanism for glucocorticoid (Gc) regulation of POMC in the pituitary, the effects of Gcs on hypothalamic POMC are controversial. In obese rats, adrenalectomy

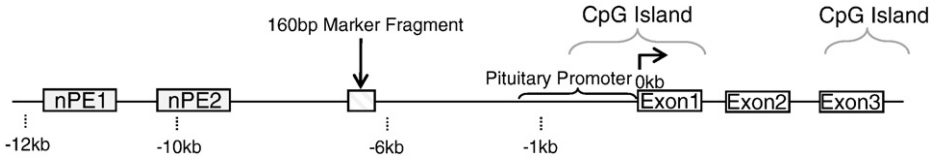

Fig. 3. The human POMC gene promoter region. nPE1 = neuronal POMC enhancer 1, nPE2 = neuronal POMC enhancer 2; nPE1 and nPE2 are associated with hypothalamic transcription of POMC (de Souza et al., 2005). Position of CpG islands as defined by Newell-Price (2003). The position of the pituitary promoter region is defined by Bilodeau et al. (2006). The 160 bp marker region was used in our studies on the sheep POMC promoter (Stevens et al., 2010).

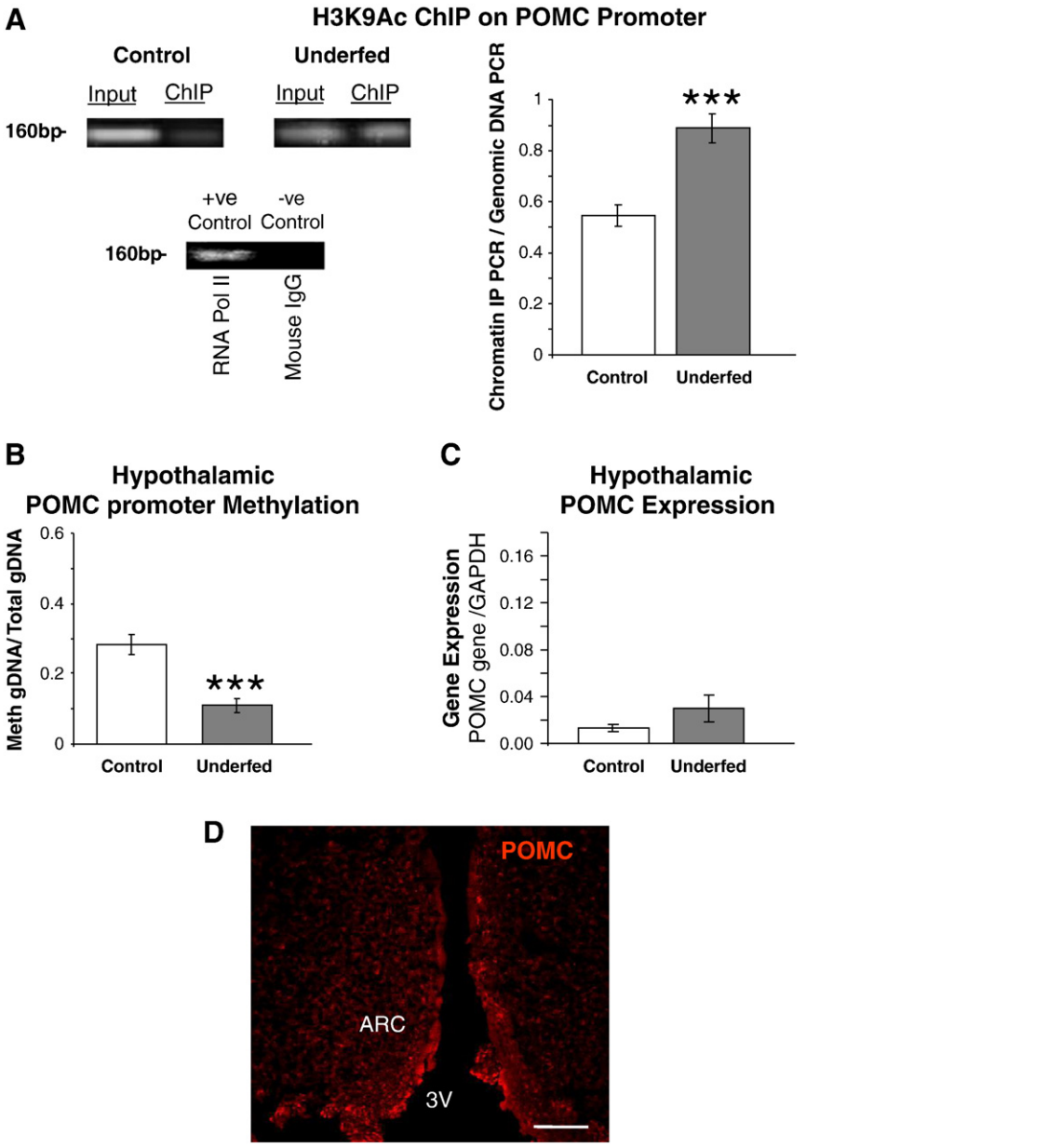

Fig. 4. Epigenetic changes associated with the POMC gene in the foetal hypothalamus. A) Chromatin Immunoprecipitation (ChIP) of acetylated histone H3K9 as a marker of transcriptionally active DNA. An antibody to RNA polymerase II was used as a positive control and mouse IgG as a negative control. Ratio of PCR signal from H3K9Ac immunoprecipitated DNA to total genomic DNA for DNA purified from gestational age day 131 ventral hypothalamic sections (Control n = 9; Underfed n = 11). B) Ratio of POMC gene promoter marker PCR signal from methylated genomic DNA to total genomic DNA for ventral hypothalamic sections at gestational age day 131 (Control n = 9; Underfed n = 11). C) Expression levels of POMC in total RNA purified from ventral hypothalamic sections at gestational age day 131 enriched for arcuate nucleus (Control n = 9; Underfed n = 11). D) Immunohistochemistry for foetal hypothalamic POMC was detected using our monoclonal antibody (A1H5 — unpublished data) and labelled with TRITC secondary antibody scale bar = 200 μm, 3V = 3rd ventricle, ARC = arcuate nucleus, *** = P<0.001.

(ADX) resulted in a reduction in food intake and body weight suggesting Gcs may increase food intake. Furthermore the above observations were reversed following the administration of Gcs (Strack et al., 1995). When normal rats were continuously administered Gcs for three days, there was an increase in food intake and body weight (Zakrzewska et al., 1999). One possible explanation is that Gcs down-regulate the anorexigenic peptide POMC, which would increase food intake (Strack et al., 1995). In addition, Gcs suppress food intake, by inducing the release of insulin, and therefore stimulating POMC neurones (Strack et al., 1995). Gcs can also directly increase POMC expression in the hypothalamus (Wardlaw et al., 1998). Thus, Gcs may induce and suppress food intake in different experimental paradigms to control appetite regulation and indeed they can act by regulating other neuropeptides including neuropeptide Y (Fig. 1). Therefore in the hypothalamus, glucocorticoid mediated regulation of POMC and other neuropeptides could be influenced by epigenetic changes in the glucocorticoid receptor.

Evidence of modifications of the epigenetic status of the glucocorticoid receptor, were found in the liver from the offspring of rats that were fed a protein restricted diet throughout pregnancy. When compared to the control offspring, the diet restricted offspring had decreased glucocorticoid receptor methylation, with a 200% increase in glucocorticoid receptor expression. The increase in glucocorticoid receptor expression suggests increased influence of Gcs. Furthermore the changes in glucocorticoid receptor methylation persisted in the offspring even though the dietary restriction had stopped suggesting that the methylation status of genes is potentially permanent (Lillycrop et al., 2005). This study indicates that maternal

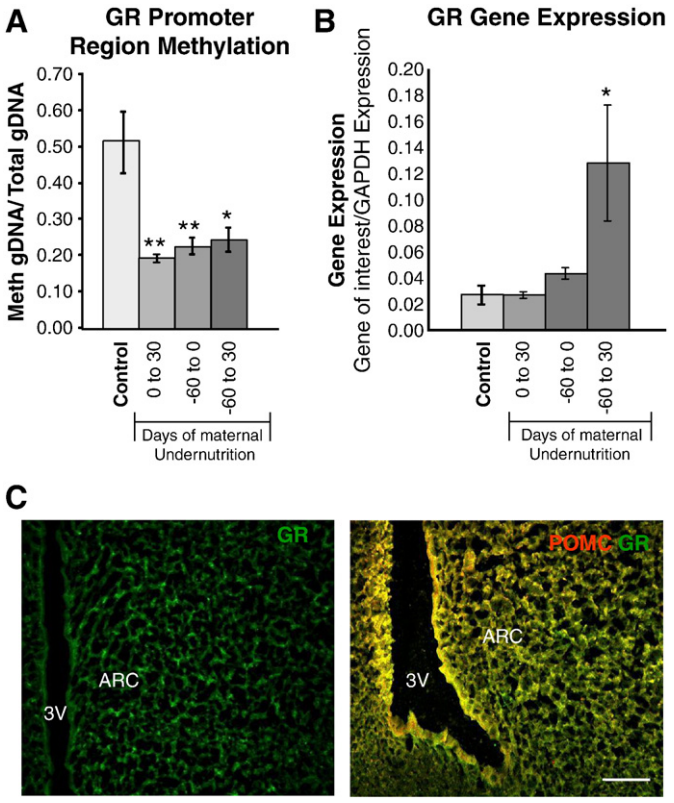

Fig. 5. The effect of different periods of periconceptional undernutrition on hypothalamic GR, methylation and gene expression. The −60 to +30 group (UN −60 to +30) were underfed from 60 days before conception to 30 days after conception; the −60 to 0 group (UN −60 to 0) were fed the same diet as the −60 to +30 group but were allowed to feed ad libitum from conception; the 0 to +30 group (UN 0 to +30) were fed the same diet for 30 days after conception. A) Methylation of GR promoter marker in hypothalami from controls (n=7), −60 to +30 (n=9), −60 to 0 (n=8) and 0 to +30 (n=7) feeding regimens. A marker of the GR gene promoter region CpG islands was used to compare the ratio of methylated to unmethylated DNA. One-way ANOVA with Tukey HSD post hoc test compared with control group *P<0.05, **P<0.001. B) Transcriptional expression of GR in the hypothalami from controls (n=7), −60 to +30 (n=11), −2 to +30 (n=7) and −60 to 0 (n=8) feeding regimens. One-way ANOVA with Tukey HSD post hoc test compared with all other groups*P<0.05. C) Immunohistochemistry for foetal hypothalamic GR was detected using the primary antibody M20 (Santa Cruz) and then targeted with a FITC labelled secondary antibody. Immunohistochemistry showing distribution of GR in the foetal sheep hypothalamus and the colocalisation of POMC and GR in the foetal sheep at 131 days of gestation. Magnification = 10×. Scale bar = 200 μm, 3V = 3rd ventricle, ARC = arcuate nucleus.

nutritional insults can alter the methylation patterns of glucocorticoid receptor in the foetus.

We have shown a glucocorticoid receptor gene promoter region marker to be associated with foetal programming of the hypothalamus in response to maternal undernutrition. This marker is situated immediately 5′ of the glucocorticoid receptor exon 1 complex (Stevens et al., 2010). Our observations are supported by increasing evidence for the role of the glucocorticoid receptor gene exon 1 promoter regions in modulating both the activation and the repression of glucocorticoid receptor gene expression through glucocorticoid response units (GRUs) (Geng et al., 2008) and the association of the NGFIA transcription factor with the maternal programming of stress (Weaver et al., 2004).

5. Discussion

There is considerable evidence of a role for hypothalamic POMC in the control of food intake and energy balance as seen with the effects of loss of function mutations in POMC and in the melanocortin MC_4 receptor (Pritchard et al., 2002; Pritchard and White, 2007). However these mutations cannot account for the marked expansion in the development of obesity. The reasons for this are multifactorial, not least of which is the increasing availability of food both at the time of birth and later in life. However the evidence for inter-generational programming is also a concern given the strength of the data from epidemiological analysis. Although this is based on studies of famines, many women in western society are dieting at the time of conception. Therefore it is important to understand how under-nutrition in the mother might impact on the foetus and the outcome for the off-spring.

The concept that maternal insults cause epigenetic changes in promoter regions of genes in the foetus is evidenced by the methylation of the glucocorticoid receptor promoter in the foetal brain in models of maternal stress (Seckl and Holmes, 2007). Therefore we put forward the hypothesis that there could be epigenetic changes in the promoter region of hypothalamic neuropeptides in offspring from mothers who had been under-nourished around the time of conception. We found hypomethylation of a promoter region of POMC, which could result in changes persisting into adulthood and which could alter the way the gene responds to counteract increased food intake. This was a very specific effect in that we did not identify any epigenetic modifications in neuropeptide Y nor did we find any changes in methylation or acetylation in the POMC promoter region in the pituitary (Stevens et al., 2010).

Interestingly while considering the glucocorticoid receptor, we were surprised to find epigenetic changes in its promoter consistent with up-regulation of the receptor. This occurred concomitantly with increases in glucocorticoid receptor gene expression even at the foetal stage of the offspring.

The observation of hypomethylation occurring in both POMC and glucocorticoid receptor genes after maternal undernutrition in the sheep provides striking evidence for epigenetic changes which could act as a programming mechanism that predisposes hypothalamic feeding centres to abnormal regulation later in life. Indeed mature sheep that had undergone a similar phase of maternal undernutrition were found to have increased body weight (Rumball et al., 2009).

Programming would be expected to decrease POMC gene expression if there is a resultant increase in obesity as predicted in the study by Delahaye et al. (2008). Although we did not find a change in POMC gene expression in line with previous work (Ikenasio-Thorpe et al., 2007), it may be that this only occurs later in life, supporting the 'thrifty phenotype hypothesis'. The hypothesis suggests an adaptive response by the foetus to undernutrition allowing an increased chance of postnatal survival (Kapoor, 2006; Seckl, 2004). However, if such changes persist into adulthood where food is abundant, a defect in normal appetite regulation may subsequently lead to over-eating and obesity. This may explain numerous findings that prenatally under-nourished animals are hyperphagic when given hypercaloric or high fat diet postnatally, compared to control animals (Vickers et al., 2000).

The epigenetic changes in the glucocorticoid receptor which result in over-expression of the glucocorticoid receptor could be more relevant to regulation of the neuronal pathways in the hypothalamus, as Gcs are known to influence many of the pathways. It would be necessary to predict that Gcs down-regulate POMC gene expression in the hypothalamus. However this appears to be very dependent on the experimental setting.

In conclusion, there is sound evidence of a role for epigenetics in the programming of hypothalamic neuropeptide pathways leading to an increased propensity for obesity in the adult off-spring. More work is required to understand the mechanisms and to identify approaches to reverse these changes so that the normal homeostatic balances can regulate food intake.

Acknowledgements

Support has been gratefully received from the NIHR Manchester Biomedical Research Centre, the Health Research Council of New

Zealand, the National Research Centre for Growth and Development, New Zealand, and the Canadian Institutes of Health Research.

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