Tissue specific epigenetic differences in CRH gene expression

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1. ABSTRACT

Corticotropin Releasing Hormone (CRH), a 41-amino acid peptide, is a major regulator of hypothalamic-pituitary-adrenal axis function. CRH also has important roles in several processes pertaining to pregnancy and parturition, including being a possible regulator of gestational length and predictor of pre-term birth. Regulation of the CRH promoter exhibits some tissue-specificities, the most well characterized example being glucocorticoids, which can stimulate placental CRH production but suppress hypothalamic CRH. In the last decade there has been growing interest in the role of epigenetic regulation of gene expression. Modification of the structure of chromatin is an example of epigenetic change affecting gene expression. We have found that inhibition of histone deacetylases results in an increase in CRH expression in the AtT20 pituitary cell line, but a decrease in CRH expression in the placenta. In this paper we review tissue specific differences in CRH gene expression, and discuss how epigenetic chromatin modification mechanisms can relate to tissue specific differences in expression of CRH.

2. INTRODUCTION

Corticotropin Releasing Hormone (CRH) is a 41-amino acid peptide originally identified within the parvocellular neurons of the paraventricular nucleus of the hypothalamus where it has been shown to act as a major regulator of hypothalamic-pituitary-adrenal (HPA) axis function (1). The HPA axis constitutes a major pillar of the stress response and dysregulation of this system has been associated with pathologies such as anxiety and depression (2, 3).

In the years following its characterization, CRH and its receptors have been identified at multiple extrahypothalamic sites both within the central nervous system including the cerebral cortex, basal ganglia, hippocampus, amygdala, thalamus and spinal cord, as well as in peripheral tissues including the skin, the heart, ovaries, testes, myometrium, decidua, endometrium and in the placenta (4-17).

Placental CRH production is restricted to humans and higher primates and has been implicated in a number
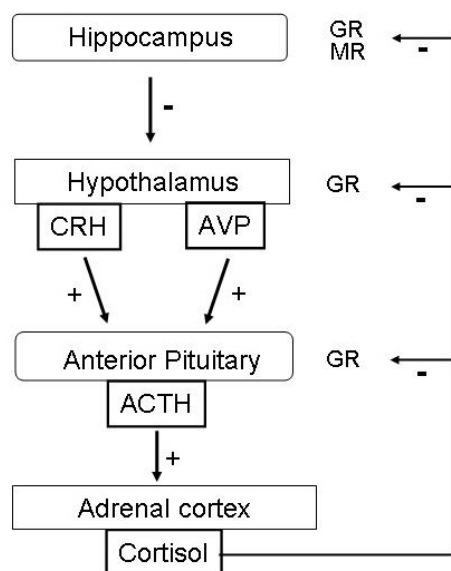
Figure 1. Schematic diagram of the hypothalamic-pituitary-adrenal (HPA) axis**.** Parvocellular neurons from the hypothalamus release corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP) which stimulate adrenocorticotropic hormone (ACTH) secretion from the anterior pituitary. ACTH stimulates the release of cortisol from the adrenal cortex, which has a number of biological functions involved in the stress response. Cortisol exerts a negative feedback on the hypothalamus, anterior pituitary and most importantly via the hippocampus. GR=glucocorticoid receptor; MR=mineralocorticoid receptor.

of processes pertaining to pregnancy and parturition (18-21). Importantly, placental CRH has been considered to be a possible regulator of gestational length, providing a critical link to premature delivery (22). CRH has been proposed to act directly on the myometrium to regulate contractility via CRH receptor isoforms which link to various second messenger systems (23) and also to act on the fetal adrenal to drive fetal adrenal DHEA production and consequently increases in estriol and the onset of labor (24).

Understanding the regulation of CRH expression is therefore a crucial step towards understanding key physiological processes such as the regulation of the stress response, the process of parturition and the pathophysiology of depression and preterm delivery. The CRH promoter exhibits a number of tissue-specificities with regards to its regulation, the most well characterised example is that of glucocorticoids which stimulate placental CRH production but suppress hypothalamic CRH (9, 25).

The role of epigenetic systems in gene expression is increasingly being appreciated as an important regulator of transcriptional control in many systems including the placenta (26-29). In this paper we begin an exploration of the impact that the epigenetic

acetylation modification of histones has on the regulation of the CRH gene in the placenta compared to the hypothalamus.

3. CRH AND THE HYPOTHALAMIC-PITUITARY-ADRENAL (HPA) AXIS

CRH plays an important role in coordinating the activity of the hypothalamic-pituitary-adrenal (HPA) axis which represents one of the body's major responses to homeostatic challenge (30, 31). CRH is synthesised in the parvocellular neurons of the hypothalamus and these neurons receive inputs from both limbic and brain stem centres allowing them to respond both to physical and psychological stressors (3). CRH is released in conjunction with arginine vasopressin (AVP) into the hypophyseal portal system where they act synergistically to stimulate release of adrenocorticotropic hormone (ACTH) from the corticotroph cells of the anterior pituitary (3, 30, 31). Furthermore, CRH and AVP also each act to reciprocally stimulate the release of the other (8).

In non-stressful situations CRH and AVP are secreted in a pulsatile circadian rhythm with peak secretion early in the morning (8). In situations of stress the amplitude and synchronisation of the CRH and AVP response is increased, leading to a surge in ACTH which culminates in the release of glucocorticoid mediators such as cortisol from the adrenal cortex (8, 30, 31). Glucocorticoids have a number of physiological functions pertaining to glucose metabolism, immune function, inflammatory response, fluid and electrolyte balance, vascular tone, muscle and bone metabolism, growth and development as well as central nervous system effects (30).

Glucocorticoids also act to regulate their own production by exerting a negative feedback effect on the hypothalamus and anterior pituitary to suppress CRH and AVP release (Figure 1). This glucocorticoid negative feedback control occurs via a dual receptor system consisting of the glucocorticoid receptor (GR) and mineralocorticoid receptor (MR) in the hippocampus (32).

4. CRH AND THE PLACENTA

The presence of CRH within the human placenta was first identified in 1982 (13), and has subsequently generated a great deal of research interest in defining the role and significance of CRH during pregnancy and parturition (19). Production of CRH by the placenta occurs in the syncytiotrophoblast and intermediate trophoblast cell layer of the placenta, and CRH produced by the placenta is identical with respect to structure, immunoreactivity and bioactivity to that produced by the hypothalamus (33). Placental CRH has been found to have multiple local regulatory functions, such as in the maintenance of blood flow, through its ability to induce vasodilation in the uterine and fetal placental vessels via signalling through the NO/cGMP pathway (12, 34).

Placentally derived CRH is predominantly secreted into maternal plasma, however a proportion also
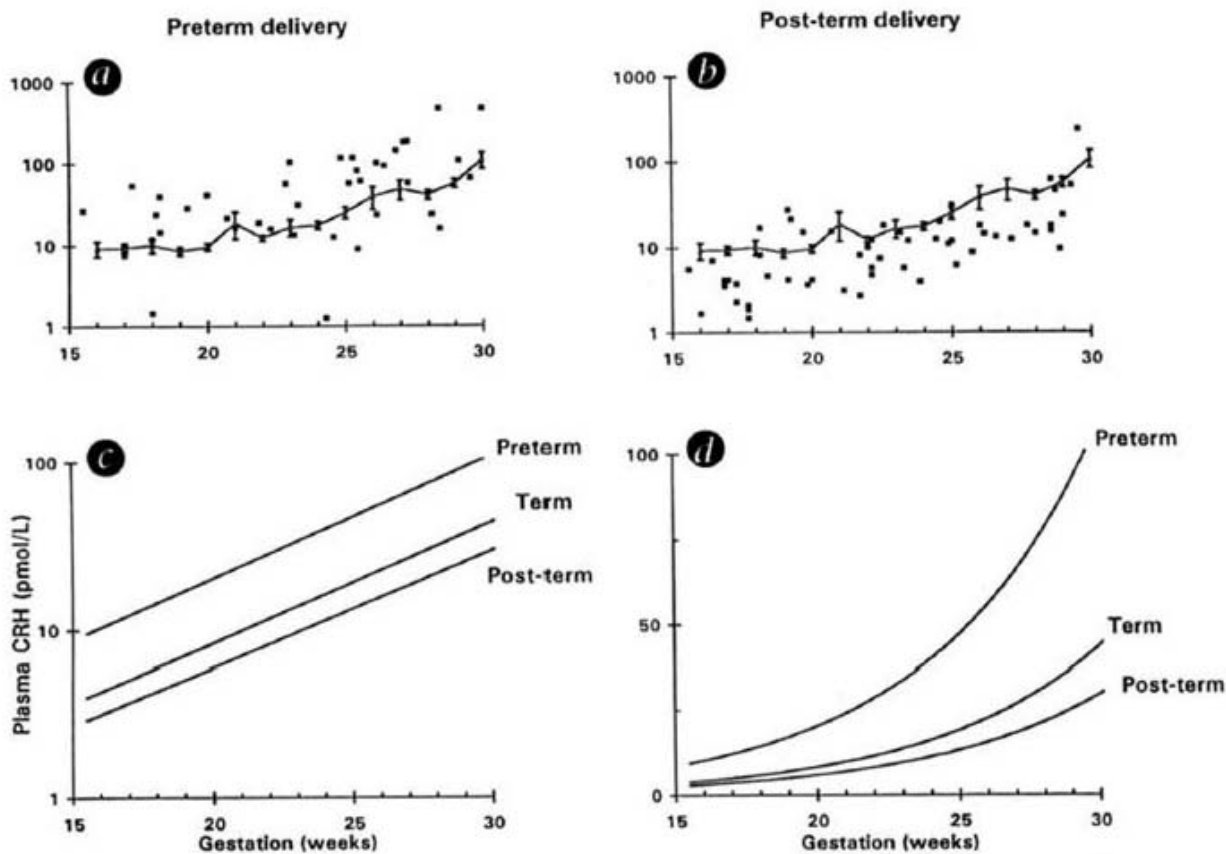
Figure 2. Maternal Plasma CRH levels throughout mid-gestation. CRH levels in women whose pregnancies ended in spontaneous pre-term labour (n=24), term labour (n=308) or post-term delivery (n=29). *a,* Mean plasma CRH of women delivering at term (connected data points with error bars (+/- standard error)) compared to women delivering pre-term (individual measurements), *b* Mean plasma CRH of women delivering at term (connected data points with error bars (+/- standard error)) compared to women delivering post-term, *c,* log linear regression curves fitted to plasma CRH results for the three groups, *d* Non-logarithmic representation of regression curves demonstrating the exponential increase in CRH. Figure adapted from McLean *et al* (22).

enters the fetus where it is able to stimulate the fetal pituitary leading to release of cortisol from the fetal adrenal gland (18). Cortisol reciprocally stimulates placental CRH production and also induces fetal lung maturation (18, 35). In addition to stimulation of cortisol from the fetal adrenal, placental CRH is also able to stimulate the fetal zone of the adrenal to produce dehydroepiandrosterone sulphate (DHEAS) the precursor to estrogen, which is then converted to estriol by the placenta (36, 37). Interestingly, the fetal zone of the adrenal involutes after delivery suggesting that it is maintained by placental factors, such as CRH (18).

In 1995 a prospective longitudinal study monitoring maternal plasma CRH levels at different stages of gestation following a cohort of 500 women (Figure 2) found that maternal plasma CRH reached concentrations of approximately 800pg/mL, and can be as much as 1000-fold greater than non-pregnant women (22, 34, 38). Those findings were analysed using linear regression analysis, and of great clinical significance

was the observation that maternal plasma CRH concentrations rise exponentially with length of gestation, accelerating rapidly after 25 weeks and peaking at the time of delivery (22). Furthermore, women who experienced spontaneous premature delivery had elevated CRH levels detected during the second trimester, while low CRH levels were associated with post-term delivery. These findings led to the hypothesis that CRH acts as a placental clock controlling the length of gestation and that a vital step in the initiation of parturition involves maternal CRH concentrations rising to a critical threshold in the final weeks of pregnancy (22, 39).

Premature birth is responsible for a range of adverse neonatal outcomes, including involvement in 50 percent of cases of cerebral palsy as well as chronic lung disease, severe brain injury, retinopathy of prematurity, necrotising enterocolitis and neonatal sepsis (18, 40). Additionally preterm babies are at greater risk of chronic complications including motor and sensory impairment, learning difficulties and behavioural problems (40).
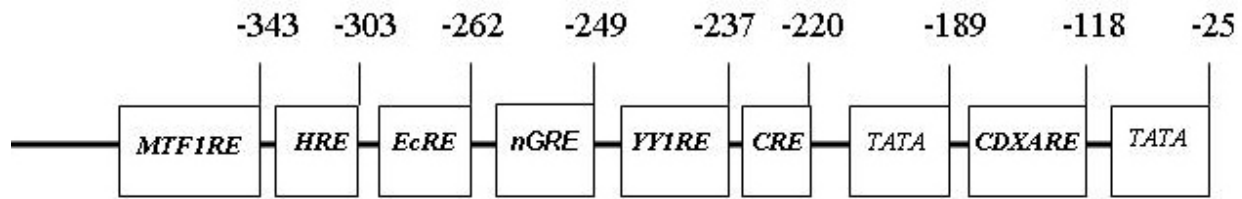
Figure 3. Diagrammatic representation of the human CRH promoter. Location of transcription factor binding sites are shown schematically. For additional discussion of these sites see King *et al* (9).

The observed association between maternal plasma CRH and length of gestation has important implications for clinical practice, as understanding the mechanisms that program the timing of birth is essential for prediction and prevention of premature delivery. In view of the severe complications surrounding preterm birth there has been great interest in characterizing the molecular control mechanisms surrounding the onset of parturition in the hope of one day being able to manipulate these systems to intervene and prevent these premature deliveries.

5. REGULATION OF THE CRH GENE PROMOTER

The CRH gene is a single copy gene located on the long arm of chromosome 8 (8q13), and consists of a promoter sequence, one intron and two exons (9). The promoter sequence of the CRH gene is well conserved across species with 97 percent homology in the first 270 bps across human, sheep, mouse and rat (9). Transcriptional regulation of gene promoters is mediated via a variety of interactions between DNA binding proteins and specific DNA regulatory elements commonly located in gene promoter regions upstream to transcription start sites (41). Given that there is only one CRH gene with a single promoter, the differential tissue specific expression of this gene must in part be due to differences in the transcription factors influencing response elements in the promoter, these differences can include the actual transcription factors present, splice variants, post-translational modifications and differences in activation pathways (9).

5.1. Role of cyclic adenosine monophosphate (cAMP)

The role of cAMP in stimulation of the CRH promoter is one of the best characterised regulatory systems affecting CRH expression. CRH expression in both the hypothalamus and the placenta has been found to be stimulated both by forskolin (an activator of adenylyl cyclase) and by cAMP (33, 42). Several endogenous agents including interleukin-1, angiotensin II, oxytocin, arginine vasopressin, norepinephrine and acetylcholine have been found to stimulate CRH expression in the hypothalamus and placenta via activation of a cAMP-dependent pathway (43-45).

cAMP acts predominantly via a cAMP responsive element (CRE) located between -228 and -220bp in the CRH promoter (Figure 3), but there is also a caudal type homeobox response element (CDXARE) at -125 to -118bp which responds to cAMP (41, 44).

Interestingly, analysis of promoters contained in the Eukaryotic Promoter Database found that 60 percent of human promoter sequences contained a CDXA region, the significance of this is not known (46). Whether CDX homeoproteins interact with the CDXARE in the CRH promoter is also unknown (44).

In addition to the CRE and CDXARE it appears that cAMP has effects at other sites in the promoter, deletion of regions such as the metal-regulatory transcription factor 1 response element (MTR1RE), Ecdysone response element (EcRE) and nuclear hormone receptor response element (HRE) diminish the stimulatory effect of cAMP on the CRH promoter suggesting that while the CRE is the primary response region it may be part of a larger cAMP response unit (CRU) (9).

5.2. Role of glucocorticoids

In contrast to the cAMP regulatory mechanisms described above, glucocorticoids appear to have opposite effects on regulation of CRH expression in the placenta compared with the hypothalamus (44). Glucocorticoids exert a negative feedback effect on CRH in the hypothalamus, and it has been shown that dexamethasone is able to reduce cAMP stimulation of the CRH promoter by more than 50 percent in transiently transfected AtT20 cells (47). Conversely, in the placenta glucocorticoids act to increase both basal and cAMP stimulated CRH promoter activity in transfected human primary placental cells (47).

The inhibition of CRH by glucocorticoids in transiently transfected AtT20 cells primarily occurs via a negatively regulated glucocorticoid response element (nGRE). The nGRE in AtT20 cells consists of three sites that can bind the glucocorticoid receptor and two atypical activating protein 1 (AP-1) response elements (AP1RE), mutations of either the GR or AP1RE resulted in loss of glucocorticoid mediated repression (9).

The glucocorticoid dependent stimulation of CRH in the placenta does not appear to involve the GR binding sites which mediate glucocorticoid repression of CRH activity in the AtT20 cells (33). A study to locate the region required for dexamethasone stimulation of CRH in the placenta, using transfections of luciferase reporter constructs containing progressive 5' deletions of the human CRH gene into cultured human primary placental cells, found that deletion of the region between -342 and -213bp resulted in loss of the dexamethasone responsiveness (47).

This region contains a consensus CRE, a hybrid steroid response element (HRE) and a sequence with similarities to the insect steroid hormone ecdysone response element (EcRE) (47-49). Mutation of the CRE was found to decrease basal CRH promoter activity by 30 percent and eliminate dexamethasone responsiveness (47). Conversely, mutation of the EcRE was found to induce a 2-fold increase in promoter activity and did not affect dexamethasone responsiveness. No effect was observed from mutation of the HRE.

It is clear that a functional CRE is required for dexamethasone responsiveness in placental cells, however additional studies showed that glucocorticoids and their receptor do not bind directly to the CRE, indicating that GRs stimulate the CRE by interacting with nuclear factors which can interact with the CRE (44).

Nuclear proteins binding to the CRE also show tissue specificity, as CREB and Fos proteins have been identified in complexes bound to the CRE in AtT20 cells. In contrast, CREB and cJun have been identified in CRE complexes in placental cells. Glucocorticoids are known to inhibit cFos and CREB in hypothalamic neurons and it has been suggested that these proteins may play a role in glucocorticoid inhibition of CRH in the hypothalamus (9).

6. EPIGENETICS

In the last decade there has been growing interest in the role of epigenetic systems influencing gene expression. The term epigenetics refers to stable alterations in gene expression potential which arise during cell development or proliferation (50). These are factors which alter gene expression but are not directly involved in the transcription process. Modification of the structure of chromatin is an example of epigenetic change affecting gene expression. To date, no studies have been published looking directly at the epigenetic control of CRH gene expression in either the placenta or the hypothalamus.

6.1. Chromatin structure

The basic repeating unit of chromatin is the nucleosome which consists of a histone octamer around which DNA (approximately 145-147 base pairs) is wrapped. Within the histone octamer are two copies of each histone species H2A, H2B, H3 and H4 arranged so that there is a central $(H3-H4)_2$ tetramer and two H2A-H2B dimers on each side of the tetramer. This structure is known as the nucleosome core, and these species of histones can collectively be referred to as core histones. The structure of each core histone comprises an N-terminal, globular histone fold domain and a C-terminal. The histone fold domains of the core histones facilitate both histone-histone interactions as well as the interactions between the histones and DNA (51-53).

Chromatin exists in a number of structural states, ranging from unfolded to tightly folded conformations. Remodeling of chromatin occurs either by ATP-dependent nucleosome remodeling complexes or via covalent modification of the N-terminal tails of core histones (53).

Core histone N-terminal tails can be modified by a number of processes including acetylation, deacetylation, methylation, ubiquitination, ADP ribosylation and summoylation, all of which induce some kind of conformational change in the chromatin (53, 54).

6.2. Histone acetylation and deacetylation

The enzymatic acetylation of the N-terminal tails of core histones is the most comprehensively studied post-translational modification of histones (55). Acetylation neutralises the positive charge of the target lysine, thus decreasing the affinity of the histone to the negatively charged DNA, resulting in a more loosely folded chromatin conformation. This open chromatin conformation has traditionally been thought to overcome an important physical obstacle to transcription by allowing ready access of transcription factors to their associated DNA binding site (56-59). Acetylation of the histone tail also provides a specific binding site for bromodomain containing proteins such as CBP which can assist in recruiting other transcription factors and thereby promote gene expression (53, 60).

The degree of histone acetylation is modulated by the interaction between two types of enzymes, namely the histone acetyl-transferases (HATs) and the histone deacetylases (HDACs) which are recruited to target promoters through physical interaction with specific transcription factors (Figure 4). HATs and HDACs are also able to acetylate non-histone substrates which can also play a role in transcriptional regulation (61, 62).

6.2.1. Histone Acetyl-Transferases (HATs)

Two of the most widely studied HATs, the functional homologues CBP (CREB binding protein) and p300, acetylate all core histones but preferentially acetylates H3/H4 (56, 63). p300/CBP act as co-activators for transcription and interact with a large number of factors including nuclear receptors, and the c-Jun and c-Fos transcription factors, and CREB (56, 58, 64, 65). All of those regulatory factors are known to be involved in CRH gene regulation.

6.2.2. Nuclear Receptor Co-activators

Transcriptional activation brought about by hormone signals has also been shown to be associated with the activity of HATs. Steroid receptor co-activator SRC-1 was first discovered through its interaction with the human progesterone receptor. SRC-1 is able to stimulate ligand-dependent activation by multiple nuclear receptors including the progesterone receptor (PR), glucocorticoid receptor (GR), estrogen receptor (ER), thyroid hormone receptor (TR) and the retinoid X receptor (RXR) (66, 67). SRC-1 was found to have intrinsic HAT activity in the C-terminal region with the ability to acetylate H3 and H4. SRC-1 also interacts with CBP/p300 and PCAF (52, 66).

6.2.3. Histone deacetylases (HDACs) and Histone Deacetylase Inhibitors (HDACi)

In 1996 Taunton *et al*. discovered the first histone deacetylase (HDAC) enzyme. This enzyme, known as HDAC1, had high homology to the yeast protein RPD3,
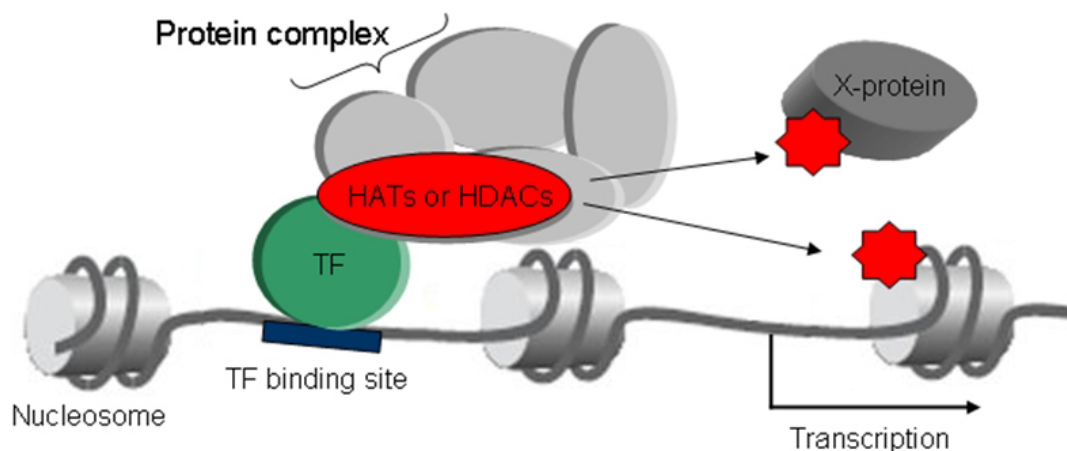
Figure 4. Model for local action of histone acetyltransferases (HATs) and histone deacetylases (HDACs). HATs and HDACs function within large multi-protein complexes ('enzymatic complex') in which other subunits are required to modify nucleosomes around binding sites. HATs and HDACs can also modify non-histone substrates ('protein X') to regulate transcription.

a known transcriptional co-repressor, providing a further link between acetylation/ deacetylation and transcriptional regulation (68).

HDACi were discovered before the isolation of the first HDACs and in many cases were used as a vehicle for purifying and cloning HDACs (69). Given the wide distribution of HDACs in mammalian tissue, it is perhaps somewhat surprising that treatment with HDACi affects the expression of only approximately 2-20 percent of genes in transformed cells (70, 71).

TSA, a naturally occurring hydroxamic acid used as a fungistatic antibiotic, was one of the earliest compounds to have demonstrated HDACi activity (70). In recent years HDACi have been developed as chemotherapeutic agents in cancer due to their ability to induce cell cycle arrest, activate apoptotic pathways and inhibit angiogenesis (72). However, it appears that the effect of HDACi is not stimulatory on all genes, as microarray profiling studies have even suggested that there are equal numbers of genes repressed by HDACi as are stimulated (73).

6.2.4. HDACs in human pregnancy

Of importance in considering the pathophysiology of preterm delivery is a study on the epigenetic regulation of human chorionic gonadotropin (hCG) and luteinising hormone (LH) receptor expression in human myometrial cells, which found that HDAC's are recruited to two GC boxes in the proximal promoter region by specificity proteins Sp1, Sp2, Sp3 and Sp-like proteins, mediating transcriptional repression (74). This effect was found to be reversed by treatment with the HDAC inhibitor (HDACi) TSA; this has great physiological relevance as hCG/LH receptor mediates the role of hCG in maintaining uterine quiescence and therefore maintaining pregnancy.

Another important study relevant to human pregnancy involves proteins involved in the cell-cell fusion

essential for the formation of the multinucleated syncytiotrophoblast layer during human placental development. The human GCMa transcription factor upregulates the expression of syncytin, a placental protein important in trophoblast fusion, via a cAMP/PKA activated pathway and involves CBP-mediated acetylation of GCMa. HDAC3 was identified as a key factor for the reversal of GCMa acetylation and HDAC3 can functionally attenuate the CBP-upregulated GCMa activity during the activated PKA signalling pathway leading to placental cell fusion. (75).

Although there are significant differences between human and rodent pregnancy it is also intriguing to note that inhibition of HDACs by TSA has the ability to significantly delay parturition in mice. (76).

7. POTENTIAL ROLE FOR EPIGENETICS IN CRH GENE REGULATION

As previously outlined, the regulation of the CRH gene is markedly different in the placenta compared with that in the hypothalamus (9) and it is possible that differing epigenetic mechanisms may contribute to the distinct expression profiles of CRH in these two tissues. Histone acetylation, has generally been associated with transcriptional activation and HDAC inhibitors (HDACi) were thought to stimulate gene expression by overcoming the repressive effect of histone deacetylation. In recent years however, it has become clear that the situation is considerably more complex; a number of genes have now been identified which defy this explanation and are in fact repressed by treatment with HDAC inhibitors (73, 77, 78). Nevertheless, it remains clear that the degree and pattern of histone acetylation are important factors influencing gene expression, and are now seen as potential contributors to tissue specific gene regulation (50, 59, 70, 79).

Studies have been conducted into the epigenetic regulation of the human growth hormone (hGH) gene
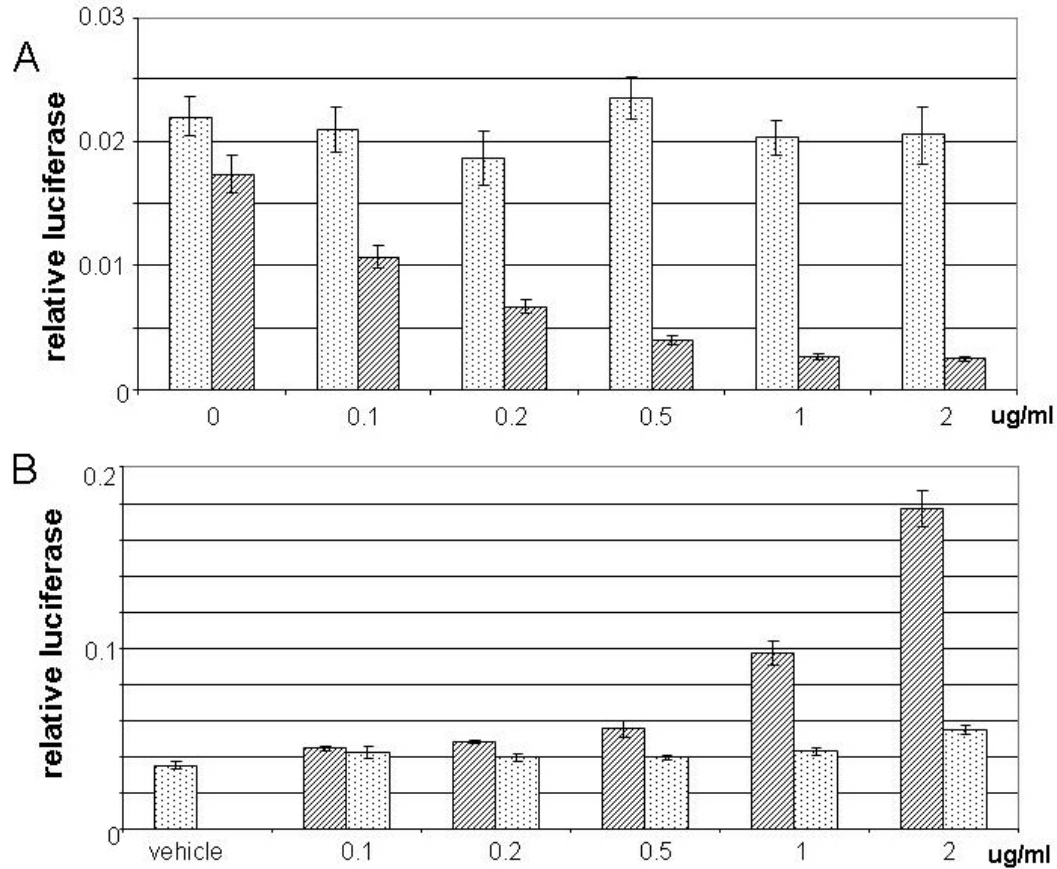
Figure 5. Effect of HDACi on CRH expression. AtT20 cells (A) or primary placental cells (B) were transfected with CRH promoter–luciferase plasmids and treated with the. AtT20 cells or Primary placental cells (cultured from term placentae after spontaneous deliveries) were treated with increasing concentrations of HDACi Scriptaid (striped bars) or a non-inhibitory analogue Nullscript (speckled bars) and co-stimulated with Br-cAMP. Relative luciferase activity representing CRH gene activity was analysed relative to vehicle treated cells. Data is displayed with error bars representing standard error.

cluster which consists of five highly conserved genes hGH-N, (hCS)-L, hCS-A, hGH-V and hCS-B which are selectively expressed in the placenta or pituitary (79, 80). hGH-N is expressed in the somatotrope cells of the anterior pituitary, the other four, (hCS)-L, hCS-A, hGH-V and hCS-B, are expressed in the syncytiotrophoblast cell layer of the placenta. ChIP assays have revealed significant differences in the patterns of acetylation and methylation of the hGH gene cluster in the two tissues. Activation of hGH-N is associated with a 32-kb domain of acetylated chromatin, this spans the entire hGH-N promoter but excludes the four placental genes. In contrast placental chromatin displays selective acetylation internal to the multigene cluster. These dramatic differences suggest that the varying acetylation patterns may play a key role in the differential tissue-specific expression of these genes (79).

Work in our group suggests that a similar phenomenon might be occurring with the CRH gene. Primary placental cells and AtT20 cells were transfected with luciferase reporter constructs containing the CRH promoter and treated with Scriptaid, a synthetic HDACi. Interestingly, opposite effects were observed whereby the

HDACi increased CRH promoter activity in the AtT20 cells but suppressed CRH promoter activity in the primary placental cells (Figure 5). This data was then confirmed using quantitative RT-PCR to examine changes in endogenous CRH gene expression in primary placental cells using TSA as the HDACi (Figure 6). This data shows indicates that CRH gene expression is differentially affected by HDACi in a tissue specific manner. Thus, CRH represents a unique model system in which to uncover the molecular mechanisms involving epigenetic regulation by histone acetylation/deacetylation.

8. POTENTIAL ROLE FOR HISTONE ACETYLTRANSFERASES/ DEACETYLASES IN CRH REGULATION

The mechanism by which an HDACi such as TSA may influence CRH expression is yet to be elucidated, however a number of known transcription factors involved in the regulation of CRH expression are known to either associate with proteins which have histone acetyltransferase or deacetylase activity or possess this activity themselves. One such example is the cAMP
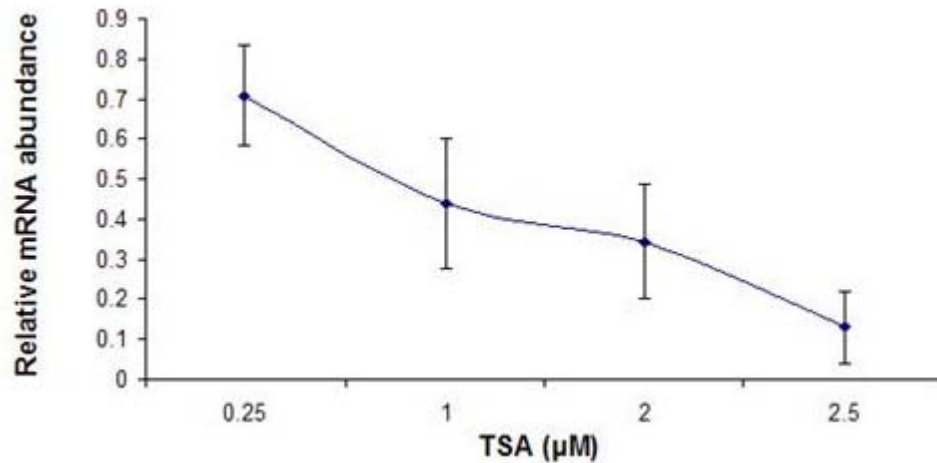
Figure 6. Effect of TSA treatment of CRH expression in primary placental cells. Primary placental cells cultured from term placentae after spontaneous deliveries were treated with increasing concentrations of the HDACi TSA and co-stimulated with Br-cAMP. Relative CRH mRNA abundance was analysed using qRT-PCR and analysed relative to human RPL13a. Statistical analysis was performed using a random effects linear regression model, data is statistically significant with p<0.01. Data is displayed with error bars representing standard error.

response element binding protein (CREB) which interacts with the histone acetyltransferase (HAT) CREB binding protein (CBP) (81). As mentioned, CREB plays an important role in stimulation of CRH expression by mediating the stimulatory effect of cAMP (43, 47). Stimulation of CRH by cAMP involves activation of protein kinase A (PKA) which catalyses the phosphorylation of CREB (9, 43, 82). Phosphorylated CREB is capable of binding to and stimulating the cAMP response element (CRE) in the CRH promoter (44).

CBP binds specifically to the phosphorylated version of CREB (81). Plasmid reporter constructs in F9 tetracarcinoma cells (which require exogenous CREB to mediate expression of CRE reporter genes) found that exogenous CBP induced a 90-fold increase in promoter activity in CRE containing genes, this is compared to the 15-fold increase observed with CREB alone. CBP however is not able to stimulate gene expression in the absence of CREB (81). As CBP cannot bind directly to DNA, association with DNA-bound activators such as CREB may also confer its histone acetyltransferase activity (63).

CBP is able to acetylate all core histones and has also been shown to associate directly with human P300/CBP-associated factor (PCAF), another known histone acetyltransferase (63, 81). It has been suggested that recruitment of CBP and PCAF to specific promoters via protein-protein signalling could represent a possible mechanism for targeted acetylation of specific regions of chromatin (63). However, whether the function of CBP in regulation of CRH expression requires its histone acetyltransferase activity is not known.

P300/CBP has also been shown to interact with another regulator of CRH expression, YY1 (83). The CRH promoter contains a YY1 response element (YY1RE) in the region of -213 to -248bp (9). King *et al* explored this

region by mutational analysis, and found that deletion of the MTF1 response element (MTF1RE), nuclear hormone response element (HRE) and ecdysone response element (EcRE) led to loss of function of the CRE (9). However, restoration of CRE function occurred with deletion of the YY1RE, implying that the YY1RE inhibits cAMP stimulation of CRE.

A study on the epigenetic regulation of the proto-oncogene *c-Myc* found that P300, which is known to function as a transcriptional repressor of this gene independent of its histone acetyltransferase ability, exerts its effect on *c-Myc* by cooperating with HDAC3 and YY1 (83). This was studied in the endogenous *c-Myc* promoter by over-expressing P300 in quiescent human MCF10A cells and then performing ChIP assays to investigate protein binding to specific regions of the DNA. Those investigations found that in cells with overexpressed P300, occupancy of YY1 and P300 increased 10- and 5-fold respectively in the YY1 binding region compared to control cells (83). Furthermore, HDAC3 occupancy increased 20-fold while there was no change in the occupancy of HDAC1 or HDAC2. The YY1 binding region was also the only region in which P300 or HDAC3 occupancy was increased compared to controls. Knock down studies using small hairpin RNAs (shRNAs) specific for YY1, P300 or HDAC3 found that knockdown of P300 led to a 12-fold induction of the promoter, while knockdown of either YYI or HDAC3 each led to 6-fold induction of promoter activity. That study also examined whether these effects were indeed a function of decreased chromatin acetylation, and found via ChIP analysis that downregulation of P300 and HDAC3 both led to an increase in H3 and H4 acetylation by 3-5 fold in the regions examined (83). This demonstrates the way in which multiple complexes can interact to produce changes in chromatin structure and therefore gene expression. The identification of an YY1 site involved in regulation of the

CRH gene is further suggestion of a role for YY1 and HDACs in controlling CRH expression (9).

9. PERSPECTIVE

In summary, the action of HDACs appears to be an important regulatory factor in the tissue specific expression of the CRH gene. HDACi suppresses endogenous CRH expression in the placenta in a dose response relationship. This finding defies the traditional theory of histone acetylation being associated with transcriptional activation and, most interestingly, epigenetic modifications of chromatin, in the form of histone acetylation and deacetylation of the CRH gene, result in opposite effects on expression of that gene in the placenta compared to the hypothalamus.

Inhibition of histone acetylation offers a potential mechanism for suppressing placental CRH, overproduction of which has been implicated in preterm delivery (22). Involvement of CRH has been suggested at multiple levels of the parturition cascade and importantly, as high CRH levels as early as the second trimester are thought to be indicative of a high risk of preterm labour, this hormone provides an opportunity for early detection and intervention (18, 20, 22, 84).

Furthermore, the potential role for HDACi in treatment of preterm delivery goes beyond suppression of CRH production. Following the work by Phillips *et al* which found that TSA reversed transcriptional repression of LH/hCG receptors by HDAC 1 and 2 in the human myometrium, Moynihan *et al* conducted a study which looked at the effect of TSA on myometrial contractility (74, 85). They examined myometrial samples obtained from C-section which were mounted under tension in organ baths and allowed to spontaneously contract or alternatively were induced to contract by stimulation with oxytocin. This study found that TSA, along with the HDACi valproic acid (VPA) and suberic bishydroxamate (SBHA), inhibited myometrial contraction by a mean maximum inhibition (MMI) of 46 percent in spontaneous test strips and 56 percent in oxytocin induced test strips suggesting that HDACi may have a role in stopping preterm contractions.

This potential for clinical application is made even more attractive in light of the fact the HDACi are already being used in a number of phase II clinical trials as a chemotherapeutic agent in a variety of haematological and solid tumours, furthermore the HDACi vornistat is already approved in clinical practice for treatment of T-cell lymphoma (85, 86). Other therapeutic uses for HDACi are also being investigated, including in treatment of inflammatory and autoimmune disease (86, 87).

10. ACKNOWLEDGMENTS

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