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Advances in understanding corticotrophin-releasing hormone gene expression

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1. ABSTRACT

Glucocorticoids inhibit corticotrophin-releasing hormone (CRH) gene expression in the hypothalamic paraventricular nucleus (PVN), but stimulate expression in the placenta. In AtT20 cells (a model of PVN CRH production) cAMP produces a high level of promoter activity. Cyclic AMP stimulation occurs through the cAMP response element (CRE) and the caudal type homeobox protein response element (CDXARE). The CRE acts as part of a cAMP response unit that includes the hybrid steroid response element (HRE), ecdysone response element (EcRE), metal-responsive transcription factor-1 response element (MTFRE), ying yang 1 response element (YY1RE) and negative glucocorticoid response element (nGRE). Cyclic AMP acts on the HRE, EcRE and MTFRE to block YY1RE mediated inhibition of the CRE. Glucocorticoids acting at the nGRE inhibit cAMP activation of the CRE. In placental cells the CRH promoter has low intrinsic basal activity and cAMP causes a modest increase in activity. Stimulation by glucocorticoids and cAMP and inhibition by estrogen and estrogen receptor alpha occurs through the CRE. In AtT20 cells multiple response elements coordinate a response to cAMP and glucocorticoids while in placental cells the CRE acts in isolation. These differences in promoter function lead to responses that meet specific physiological needs.

2. INTRODUCTION

CRH was first identified in the hypothalamic PVN where it orchestrates the hypothalamic/pituitary/adrenal stress axis (HPA) (1-3). However, CRH has now been identified in many other sites in the central nervous system where it acts as a neuro-transmitter (4-6) and in peripheral tissues including the adrenal medulla, ovary, testis, heart, lung, liver, stomach, duodenum, pancreas, T-lymphocytes, skin and placenta (4, 5, 7-11). The role of CRH in the HPA and the placenta are the focus of considerable research, but the role of CRH in many of the other tissues has yet to be elucidated.

Abnormal CRH gene expression in tissues has been associated with various pathologies. In the hypothalamus, insufficient CRH production is associated with adrenal insufficiency. In the placenta, increased production of CRH is found with pregnancy induced hypertension, intra uterine growth retardation, foetal asphyxia, chorioamnionitis, preterm labour, and multiple pregnancies (effectively more than one placenta) (12-25). CRH levels are also increased in the synovial fluid of patients with rheumatoid arthritis and osteoarthritis (26). Therefore, an understanding of the regulation of CRH gene expression may give insights into a number of physiological and pathological processes.

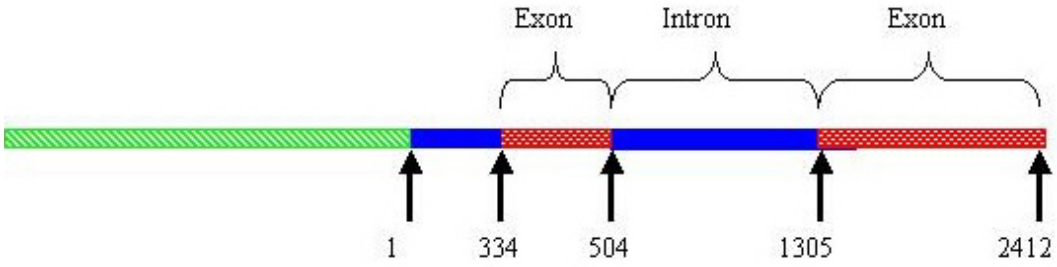

Figure 1. Schematic map of the CRH gene. The arrows and numbers refer to the base pair number in relation to the transcription start site. The blue solid bar represents the untranslated DNA (introns) and the red speckled bar represents the translated DNA (exons). The CRH promoter starts immediately upstream (5') to base pair 1 and is represented by the green striped bar.

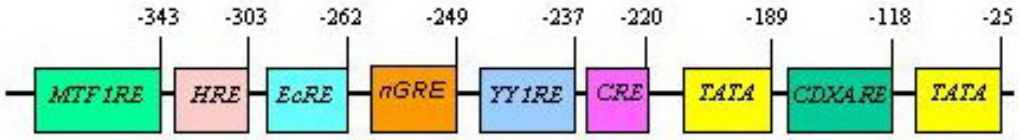

Figure 2. Diagrammatic representation of the human CRH promoter showing the sequential occurrence of response elements that are thought to have a functional role regulating gene expression in either AtT20 cells or placental cells.

3. CRH GENE AND PROMOTER STRUCTURE

There is only one gene for CRH, located on the long arm of chromosome 8 (8q13) (27). The CRH gene consists of a promoter sequence, one intron (800 bps) and two exons (582 bps) (Figure 1) (28). The gene codes for a 196 amino acid inactive pro-hormone (pro-CRH) (28). The CRH gene has only one functional promoter. There is 97% DNA sequence homology in the first 270 bps of the CRH promoter when human, sheep, mouse and rat are compared (29). This conservation of promoter DNA sequence suggests that the response elements present are important in regulating CRH gene expression across species. This suggests that the signals leading to CRH gene expression are similar across animal species and the mechanisms regulating the hypothalamic response to stress is conserved. Scanning the DNA sequence from -920 to -1 bps of the human CRH promoter with the Transfact database identified numerous consensus response elements (Figure 2) (29, 30). We have identified potential response elements for the transcription regulatory factors MTF1 (MTF1RE), nuclear hormone receptors (HRE), ecdysone (EcRE), glucocorticoid receptor (nGRE), YY1 (YY1RE), CREB (CRE), and CDXA (CDXARE).

Since there is only one CRH gene with one promoter, differences in regulation of the gene expression is in part, due to variation in the transcription factors that act on the response elements within the promoter. Variation in transcription factors can be due to differences in the type of transcription factors present, to splice variants, to post-translational modification (eg phosphorylation) or to differences in activation (eg second messenger pathways).

4. CRH PROMOTER ACTIVITY IN AtT20 CELLS

AtT20 cells, a murine corticotroph tumour cell line, do not endogenously produce CRH, are not neuronal (originating from the anterior pituitary) but they have been well characterised. These cells have been transiently transfected with CRH promoter reporter constructs and have been extensively used as a model of the hypothalamic regulation of CRH gene transcription by glucocorticoids and cAMP (31-41).

In AtT20 cells transiently transfected with CRH promoter reporter constructs, the unstimulated CRH promoter has no intrinsic activity (32, 41). This is consistent with the hypothalamus, where many neurones have no detectable CRH until they are stimulated (42-45). When the cells are stimulated with cAMP there is a dose dependent, sustained, 64 fold increase in CRH promoter activity (31, 32, 41). Phosphodiesterases have been shown to inhibit PKA pathways thereby decreasing CRH promoter activation in response to forskolin and 8-Br-cAMP in AtT20 (33). Cyclic AMP stimulates the promoter through the CRE (33), however we have demonstrated that a CDXARE is independently involved in the cAMP response (41).

Further studies of the CRH promoter in AtT20 cells have shown that the CRE does not act in isolation when it responds to cAMP. Mutation of other elements in the promoter including a HRE, a EcRE and a MTF1RE cause a reduction in the cAMP stimulation that occurs through the CRE (Figure 3). We can see that cAMP activation of the CRH promoter is significantly decreased when the MTF1RE or the HRE or the EcRE are mutated ($P<0.00017$, two tailed student's T test). Mutation of the CRE results in significantly reduced promoter response to cAMP ($P<0.0095$, two tailed student's T test) but there was still significant promoter activation consistent with the CDXARE still being functional and responding to cAMP. Mutation of the second TATA box had no effect on promoter activity.

Why the mutation of MTF1RE, HRE and EcRE led to loss of cAMP stimulation was explored by deleting

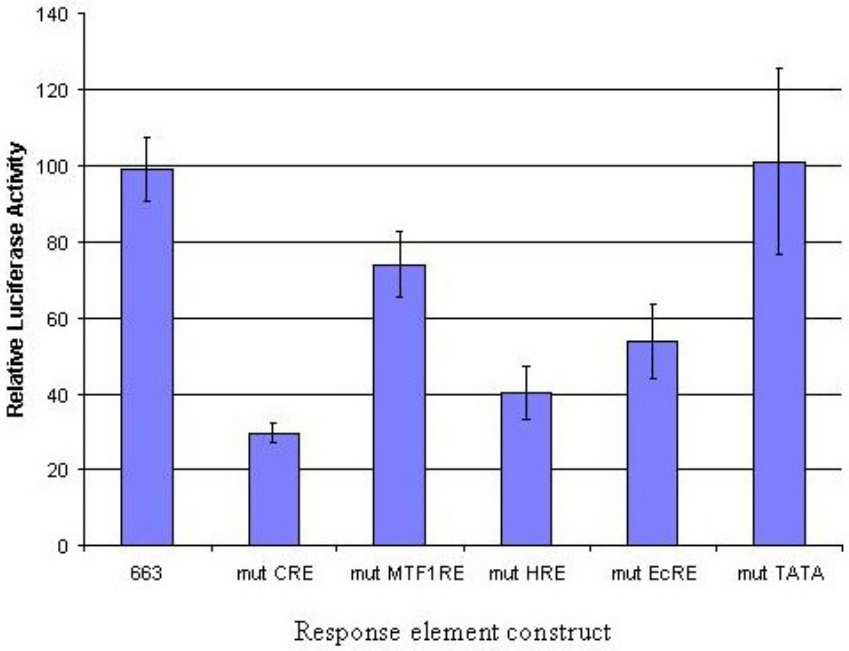

Figure 3. The role of elements between –360 and –260 bps on cAMP mediated stimulation of the CRH promoter in AtT20 cells. Site directed mutagenesis was used to individually disable the MTF1RE, HRE and EcRE of the CRH promoter. Mutation of the CRE (mut CRE) and the second TATA box (mut TATA) were used for comparison. AtT20 cells were transiently transfected with either the CRH-663, mut MTF1RE, mut HRE, mut EcRE, mut TATA or mut CRE constructs. The cells were then cultured for 24 hours in media containing charcoal stripped foetal calf serum and then treated with 3mmol/L 8-Br-cAMP and incubated for a further 24 hours. The results were standardised so that cAMP stimulated CRH-663 was given an arbitrary value of 100 and the cAMP stimulated activity of the other constructs were directly compared. All experiments were performed five times in triplicate. The error bars are 95% confidence intervals.

the region containing these elements to form the CRH-248 construct. This construct had an apparent loss of CRE function with the level of cAMP activation being the same as the CRH-213 construct which does not contain the CRE (Figure 4). The -213 to -248 bp region contains the YY1RE and the CRE. When the YY1RE was deleted (CRH-213CRE) the function of the CRE was restored so that the level of cAMP stimulated activity of the CRH-213CRE construct was the same as the full length CRH (Figure 4).

The actual level of cAMP stimulated activity was significantly higher in the CRH-213CRE compared to the CRH-248 and CRH-213 constructs ($P < 4.55E-6$, ANOVA and two tailed student's T test), but was not statistically different to the CRH-663 construct (Figure 4). These results suggest that the YY1RE inhibits the cAMP stimulation of the CRE. However, the YY1RE inhibition appears to be blocked by elements in the -663 to -248 bp region (including the MTF1RE, EcRE and HRE).

Therefore, it appears that a group of elements act as a cAMP response unit (CRU) with the CRE acting as an essential coordinating component. The CRU appears to function by cAMP stimulating the CRE, but MTF1RE, EcRE and HRE blocking the inhibitory YY1RE (Figure 5). This group of elements acts as a CRU, allowing the integration of a large number of messenger pathways resulting in a coordinated, appropriate promoter response. If the CRE and other elements acted in isolation then the promoter response to cAMP would not be as flexible. The CDXARE's response to cAMP is independent to this CRU and allows another pathway for cAMP to control promoter activity.

In AtT20 cells, when the cAMP stimulated CRH promoter is treated with physiological levels of glucocorticoids there is a dose dependent decrease in promoter activity (31, 33, 38, 40, 41). Malkoski *et al* was able to demonstrate that this inhibition predominantly occurred through a composite negatively regulated glucocorticoid response element (nGRE) (38, 40). This nGRE is composed of three regions that can bind the glucocorticoid receptor (GR) and two atypical activating protein 1 (AP-1) response elements (AP1RE) (40). Mutations of either the GR or AP1RE were associated with loss of glucocorticoid-dependent repression. Although the nGRE did not appear to explain all the glucocorticoid mediated repression, it appears to be the major site.

When we explored glucocorticoid repression of cAMP stimulated CRH promoter activity in AtT20 cells, we confirmed that the nGRE was an inhibitory element, but we also found that glucocorticoids had various effects at other sites (41). The nGRE only inhibits cAMP activation that occurs through the CRE, there is no inhibitory effect on promoter activation through the CDXARE. When the

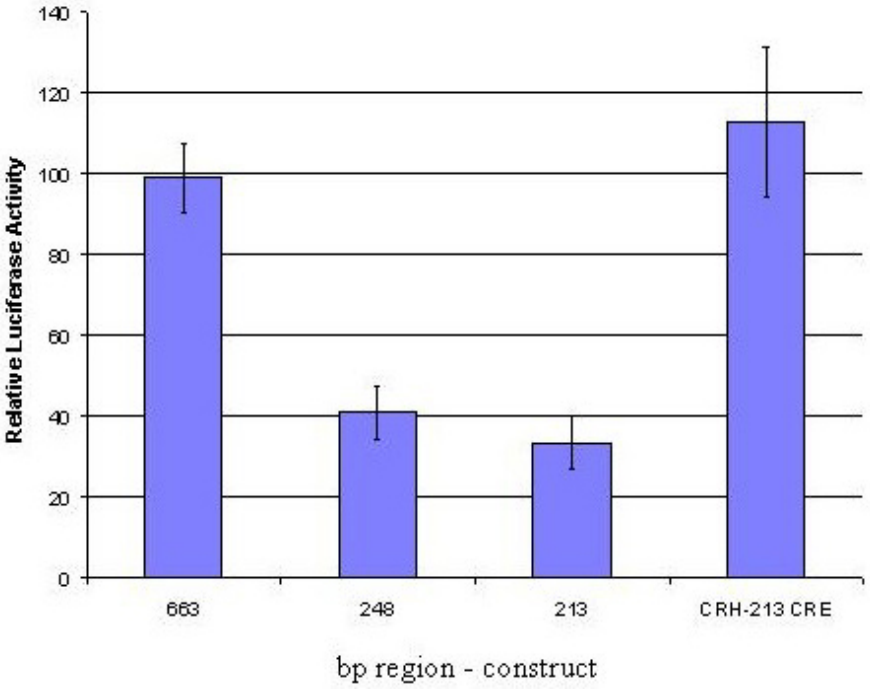

Figure 4. The effect of -213 to -663 bp region and of the YY1RE on the CRE mediated response to cAMP stimulation in AtT20 cells. The YY1RE was effectively removed by inserting the CRE 5' prime to the CRH-213 bp construct (CRH-213CRE). AtT20 cells were transiently transfected with either CRH-663, CRH-248, CRH-213 or CRH-213CRE. The cells were then cultured for 24 hours in media containing charcoal stripped FCS. The cells were then treated with 3mmol/L 8-Br-cAMP and incubated for a further 24 hours. Each experiment was performed five times in triplicate. The results were standardised so that cAMP stimulated CRH-663 was given an arbitrary value of 100 and the cAMP stimulated activity of the other constructs were directly compared. Error bars are 95% confidence intervals.

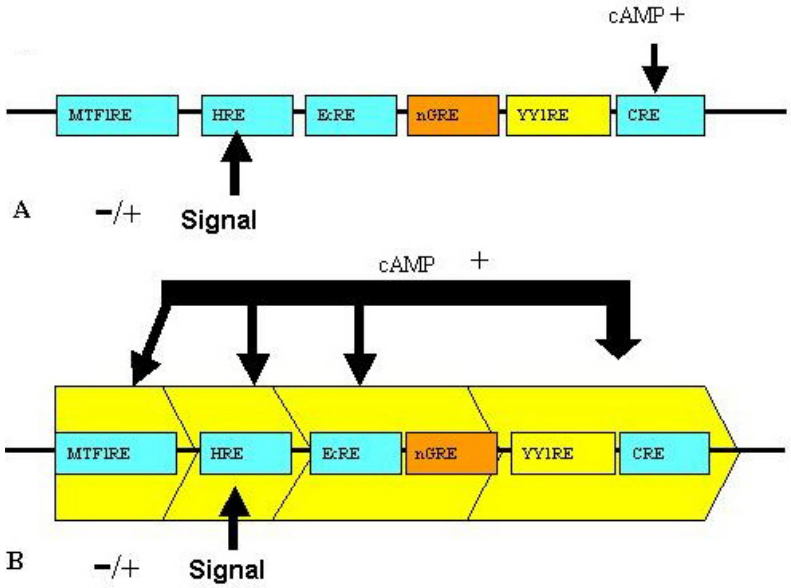

Figure 5. Elements of the CRH promoter acting as a cAMP response unit (CRU). Figure A represents the classic interpretation of the promoter response where elements are acting in isolation. The CRE will respond to the cAMP and the HRE will respond to a different message but the responses are independent of each other. In Figure B the elements are all interacting (represented by the large yellow interlocking arrows). Hence the CRE mediated response to cAMP is influenced by interactions with other element. Other message acting on the HRE directly alter the response to cAMP by influencing other elements within the response unit.

CRE was isolated from the rest of the CRH promoter, glucocorticoids alone or in combination with cAMP could stimulate promoter activity. Furthermore, the region -213 to -99 bps was also stimulated by glucocorticoids alone or in combination with cAMP. The glucocorticoid and cAMP effects at the isolated CRE or at the -213 to -99 bp region were additive (41). Further studies of these regions have suggested that the YY1RE inhibits the glucocorticoid mediated stimulation of the CRE and CDXARE (46).

These results raise a number of physiological possibilities for the CRH promoter in a system that is negatively regulated by glucocorticoids, such as the hypothalamus. If glucocorticoid levels were elevated, then limited CRH gene expression could occur when PKA pathways were activated. This would allow the PVN to maintain glucocorticoid levels and the stress response in the face of continuing insult. Furthermore, in the right context glucocorticoids may be involved in the stimulation of CRH gene expression in the PVN. Hillhouse *et al* showed that in rats, the acetylcholine mediated release of corticotrophin-releasing activity from the hypothalamus was lost for up to 4 hours after adrenalectomy (47). Plotsky *et al* has also shown that CRH mRNA response to hypotension is impaired in the absence of glucocorticoids (48). These studies suggest that glucocorticoids may not just be inhibitory but may actually be required for acute responses to stress. In the light of this, the two regions which positively responded to glucocorticoids could have physiological significance in the hypothalamic CRH stress response.

Unfortunately, there is limited data on PKC pathways in AtT20 cells because the CRH promoter has no response to PKC activators such as phorbol 12-myristate-13-acetate in these cells (33, 49). However, NPLC cells (human liver carcinoma cell line), endogenously express CRH and production is increased in response to PKC stimulation (but CRH production is not affected by PKA in these cells). This PKC stimulation is inhibited by glucocorticoids with a 90% reduction in CRH promoter activity (49). These results suggest that stimulation via PKC pathways are almost completely suppressed by glucocorticoid. Clearly it is difficult to compare results between cell types (AtT20 and NPLC cells). However, in AtT20 cells, PKA pathways stimulated at 2 regions (the CRU and CDXARE), but only the CRU was inhibited by glucocorticoids. In the NPLC cells PKC pathways were completely suppressed suggesting that stimulation may be occurring through the CRE or nGRE (which would allow complete inhibition by glucocorticoids). Hence, this suggests that a stimulus which activates PKC pathways would be suppressed by physiological glucocorticoid levels, while a different stimuli which activated PKA pathways could potentially achieve much higher circulating glucocorticoid levels due to an ability to resist inhibition.

5. CRH PROMOTER ACTIVITY IN HUMAN PLACENTAL CELLS

Placental CRH production is very different to that in the hypothalamus. The placenta produces and releases CRH constantly through the day with no apparent diurnal variation (50). Throughout a pregnancy, CRH production increases gradually but exponentially (51-53). Although placental CRH production increases in states of stress (such as pregnancy induced hypertension or intrauterine growth retardation), there is only a two to five fold increase in circulating CRH (12, 13). When compared to the hypothalamus where CRH production can change 30 - 100 fold within hours, the level of placental CRH gene expression is not prone to large variations.

In human placental cells transfected with CRH promoter/reporter constructs, we found that the CRH promoter is constitutively expressed, but this basal expression is only 2.47 fold higher than the construct with no promoter ($P = 1 \times 10^{-5}$ unpaired, two tailed Student t test) (46). At first glance, this level of promoter activity is unexpectedly low. However, the normal term placental weights 600grams and a significant percentage of the cells produce CRH. Riley *et al* used immunohistochemistry to demonstrate CRH staining in syncytiotrophoblasts, intermediate trophoblast but not in cytotrophoblast. CRH was also present in cells of the amnion and umbilical vessels (54). Although each cell only produces minuscule amounts of CRH, the combined production from all the cells is large. Therefore, if each cell was able to produce large amounts of CRH then the cumulative production from the entire placenta would be enormous and the body would be overwhelmed by this highly biologically active peptide.

Cheng *et al* used human placental cells transfected with CRH promoter/reporter constructs and found that cAMP caused a five fold increase in promoter activity while glucocorticoids caused a two fold increase in promoter activity (55, 56). When the cells were treated with cAMP and glucocorticoids together the stimulation was additive causing a seven fold increase in CRH promoter activity (55). Using promoter deletion and mutation studies Cheng *et al* demonstrated that cAMP and glucocorticoids stimulate promoter activity through the CRE (55, 56).

If we reconsider the AtT20 data, when the CRE was isolated from all other regions of the promoter, cAMP and glucocorticoids caused the same pattern of promoter stimulation that occurred in the placental cells. Furthermore, the level of promoter stimulation seen was also the same as that seen in the placental cells (41, 46).
The role of mitogen-activated kinase pathways (MAPK) in placenta has been explored by Cheng *et al* using transfected primary placental cells. In these studies they demonstrated that MAPK stimulated CRH promoter activity through the CRE and that no other element was involved (57).

In the placental cells there appear to be a number of inhibitory influences on the CRH promoter. When the EcRE was mutated there was a two fold increase in basal, cAMP stimulated and glucocorticoid stimulated promoter activity (55). Hence, the EcRE appears to be an inhibitory element in the placental cells (while in the AtT20 cells, this element was part of the CRU and mutation decreased cAMP stimulated activity). Other steroid hormones (estrogen and progesterone) can inhibit the promoter. Ni *et*

al demonstrated that estrogen inhibited basal, cAMP induced and glucocorticoid induced CRH promoter activity in placental cells. This estrogen inhibition was dependent on the alpha estrogen receptor and inhibited through the CRE (58, 59). The progesterone mediated inhibition is dependent on either the progesterone receptor A or the glucocorticoid receptor and inhibits through the CRE. The progesterone receptor B actually stimulates CRH promoter activity in placental cells (59).

From these studies it appears that the CRE alone is responsible for stimulation by cAMP and glucocorticoid or inhibition by estrogen and progesterone of the CRH promoter in placental cells (55, 56, 58-60). The only other element that appeared to have any role in CRH promoter activity in placental cells was the EcRE (55).

These data suggest that in the placenta, the CRE of the CRH promoter acts in isolation.

6. NUCLEAR FACTORS BINDING TO THE CRE OF THE CRH PROMOTER

Clearly, the ability of nuclear factors binding to the CRE to interact with other factors binding to other elements of the CRH promoter is tissue specific and appears to determine the promoter response to cAMP and glucocorticoids. We proposed that this tissue specific ability to interact with other factors on the promoter is determined by the type of nuclear factor binding to the CRE (41).

We performed electrophoretic mobility shift assays (EMSA) using the CRE and nuclear extracts from AtT20 cells and placental cells. The nuclear extract from the AtT20 cells formed three different protein complexes on the CRE. Each protein complex had different binding characteristics (determined by competition binding studies) confirming that each band was a unique protein complex and not just monomers and dimers of the same protein. Antibody supershift assays identified CREB and Fos proteins in these complexes. Furthermore, the binding of the three complexes were influenced by pre-treatment with cAMP and glucocorticoids. The three different protein complexes increased their binding to the CRE following exposure of the AtT20 cells to cAMP and when glucocorticoids were added the binding of two of the complexes decreased and binding of the third complex increased (41). Glucocorticoids have been shown to inhibit CREB and cFos activity in the PVN and this has been proposed as a mechanism for glucocorticoid mediated inhibition of CRH production (61, 62). Clearly, this is not the only mechanism involved, since the isolated CRE was stimulated by glucocorticoids in transfection studies in AtT20 cells (41). However, these changes in nuclear factor binding at the CRE may be significant in the interactions between the CRE and the nGRE in the inhibitory response to glucocorticoids.

When the nuclear extracts from placental cells were studied, we identified two unique protein complexes on the CRE. Each protein complex had different binding characteristics (determined by competition binding studies) and the binding was not affected by pre-treatment with cAMP or glucocorticoids. Antibody super shift assays identified CREB and cJun proteins in these complexes (41).

It is worth noting that AtT20 cells contain cJun, while placental cells contain Fos proteins. Why cJun was not found to bind to the CRE in AtT20 cell nuclear extracts and Fos proteins were not found to bind to the CRE in placental cell nuclear extracts is still unclear. Since the EMSA experiments discussed above used only the CRE, which was present in excess, this excludes inhibition of binding by factors at other elements in the promoter or factors binding at the CRE. This suggests that there is interference with the nuclear factors that prevents them from binding to the CRE. This could be post-translational modification (such as phosphorylation) or interference from other nuclear factors. Further research is required to define the factors involved in this apparent selective nuclear factor binding.

7. MODELLING THE FUNCTION OF THE CRH PROMOTER

Clearly, the CRH promoter is functioning differently in AtT20 cells when compared to its function in human placental cells. To help “visualise” this difference, we attempted to model the CRH promoter’s method of function in these different cell types (Figure 6 and 7). These models emphasise the major differences in the function of the CRH promoter, highlighting that the elements within the promoter are functioning in a completely different manner in the AtT20 cells compared to the placental cells.

8. TISSUE SPECIFIC CRH GENE EXPRESSION GIVES INSIGHTS INTO PROMOTER FUNCTION

The CRH gene appears to be regulated by a simple promoter. It only has the one promoter that is positioned 5' to the coding region. However, the diversity of function that is achieved is out of proportion to the apparent simplicity of the promoter. This has led to a change in thinking about how promoters function and achieve the required physiological outcome. In the late 80's and early 90's, response to a stimulus was considered as activation of a pathway which lead to activation of a specific response element in a promoter causing an alteration in gene expression. From the preceding discussion it is obvious that we need a much more complicated model to examine promoter function.

In the AtT20 cell studies discussed, activation of the PKA pathway leads to stimulation of the promoter through a CRU (of which the CRE is a central component) and a CDXARE. When the isolated CRE was stimulated by cAMP, there was a five fold induction of promoter activity, compared to the 64 fold increase that was seen in the full length promoter. This enhanced promoter activation was achieved by amplification of the CRE response by interactions with other elements of the CRU and addition of this response to the response from the CDXARE. This system allows integration of multiple messages at multiple

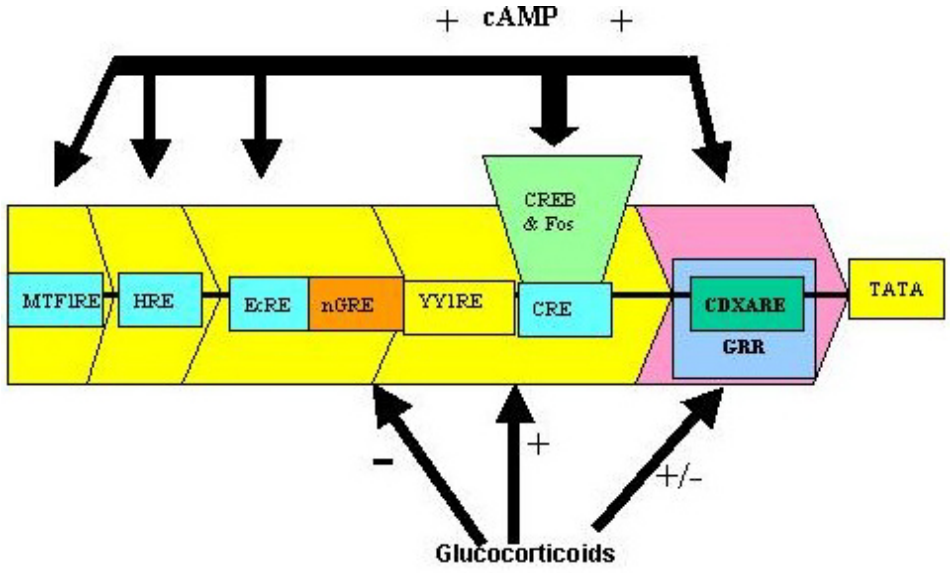

Figure 6. Model of the CRH promoter in AtT20 cells. The MTF1RE, HRE, EcRE, nGRE, YY1RE, CRE, CDXARE and the TATA box are illustrated as labeled boxes. The cAMP responsive unit is highlighted by large yellow interlocking arrows. The transcription factors, CREB and Fos are depicted as a labeled trapezoid in the cAMP responsive unit to highlight that the transcription factors play a major role in determining interactions within the response unit. The YY1RE box is the same as the surroundings showing that it has no obvious effect unless the other elements are not functioning. The CDXA and the -213 to -99 bp glucocorticoid responsive region (GRR) appear to act as a second cAMP response element. This region is highlighted in a large pink interlocking arrow to signify this region's ability to interact with the other cAMP responsive unit. The sites of action and effect of cAMP and glucocorticoids are shown with black arrows and +/- signs.

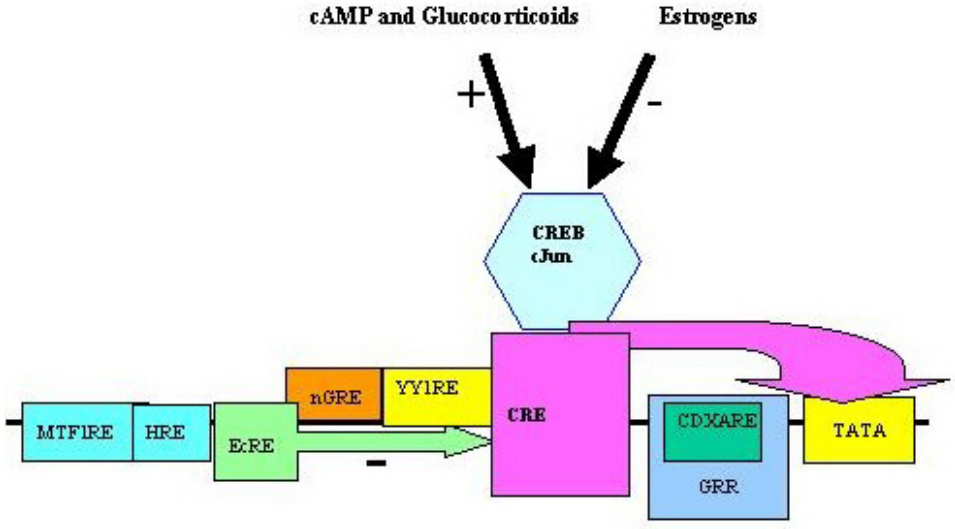

Figure 7. Model of the CRH promoter in placental cells. The CRE which was the only element identified as having a significant role in the cAMP and glucocorticoid effect on the CRH promoter are represented as a labeled box. The box is larger than the other boxes to emphasise the importance of this element. A large arrow from the CRE goes directly to the TATA box, highlighting that the other elements have no apparent effect on the response. The transcription factors, CREB and cJun are portrayed as a labeled hexagon in the cAMP responsive unit to highlight that the transcription factors have no major interaction with other elements within the CRH promoter. The stimulatory effect of cAMP and glucocorticoids is depicted as a labeled arrow. The negative effect of estrogen and its inhibition through the CRE are shown as a labeled arrow. The EcRE is included as a labeled box with an arrow to highlight the inhibitory role this element appears to have on general promoter activity. The other identified elements are included to remind the reader that they could be involved in responses to stimuli other than cAMP/glucocorticoids.

levels. If we consider the CRH promoter response to cAMP in AtT20 cells, we see that messages can influence the promoter activity through alterations to the PKA pathway, directly or indirectly on the CRE, or by effects on the other elements which are interacting with the CRE. The CDXARE could allow for a second pathway that can lead to different regulatory influences affecting the promoter activity. Hence, for this one pathway we observe multiple levels of control that allow integration of numerous other messages to result in an appropriate promoter response. This model of promoter function would work well for the hypothalamus where there is a need for a very low level of promoter activity, but which can rapidly change to very high levels of activity, and also allow for integration of other significant messages to modify the final promoter response.

However, cells from other organs need to be able to use the same promoter to achieve a different response. This appears to be achieved by expressing a different array of nuclear factors and by alternate post-translational modification of nuclear factors. These changes appear to lead to significant alterations in the function of individual promoter response elements within the promoter. For example, response elements can lose function and the promoter can totally lose the ability to achieve an action (in the case of the nGRE, loss of function will lead to the promoter no longer being inhibited by glucocorticoids). Alternatively, the loss of a response element's function can decrease or increase the level of promoter activation achieved from the stimulation by a messenger pathway (eg loss of function of the HRE and MTFRE decreased the CRU response to cAMP). Finally, a response element can be changed from stimulatory to inhibitory (the EcRE was stimulatory in response to cAMP in AtT20 cells but was inhibitory in placental cells).

In placental cells the CRH promoter behaviour was distinctly different to that seen in the AtT20 cells. The promoter was constitutively active, the response to activation by cAMP was limited, glucocorticoids stimulated promoter activity and there was a number of consistently inhibitory influences on the promoter (estrogen, progesterone and the EcRE) which would prevent excessive levels of activation. Hence, in placental cells, the CRH promoter allows consistent CRH production which is required to maintain many aspects of foetal and maternal well being, and placental and uterine function. This promoter function is achieved by the CRE acting in isolation to all other elements. When the promoter was stimulated by cAMP, there was a five fold induction of promoter activity (which is the same level of induction seen when the CRE was isolated in AtT20 cells). The promoters reduced ability to respond to stimuli such as cAMP prevents over production of biologically active CRH which would cause adverse side effects such as maternal vasodilation or excess adrenal glucocorticoid production.

9. PERSPECTIVE

CRH is a highly biologically active peptide hormone and as such it's production is tightly controlled. The regulation of gene expression is different in the many tissues that express this peptide. This tissue specific promoter regulation is determined by alterations in nuclear factors that affect response elements within the first 350 bps of the CRH promoter. This article has attempted to give insights into how this apparently simple sequence can generate a variety of complex responses in different tissues to meet physiological needs.

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