Regulation of the expression and secretion of urocortin 2 in rat pituitary

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Abstract

We previously demonstrated that urocortin 2 (Ucn 2) is expressed in the proopiomelanocortin (POMC) cells of rat pituitary. However, the regulatory mechanism of pituitary synthesis and secretion of Ucn 2 remained to be clarified. We hypothesized that hypothalamic hormones and glucocorticoids may control the expression and secretion of pituitary Ucn 2, as Ucn 2 is expressed in POMC-expressing cells in the pituitary. Thus, in the present study, we tested this hypothesis using primary culture of rat pituitary cells. The secretion of Ucn 2 from the anterior and intermediate pituitary cells was significantly increased by 50 mM KCl. In the anterior pituitary cells, corticotropin-releasing factor (CRF) increased mRNA expression levels and secretion of Ucn 2, although arginine vasopressin (AVP) did not induce any significant change

in Ucn 2 expression or secretion. Under these conditions, both CRF and AVP increased ACTH secretion, but only CRF increased the level of POMC mRNA expression. Dexamethasone inhibited Ucn 2 and POMC mRNA expression levels, while it inhibited the secretion of only Ucn 2. In the intermediate pituitary, CRF increased both the mRNA expression levels and secretion of Ucn 2. Furthermore, dopamine did not affect either the mRNA expression level or secretion of Ucn 2 although it inhibited β -endorphin secretion in the intermediate pituitary cells. These results suggest that the mRNA expression and secretion of Ucn 2 in POMC cells of the pituitary are positively regulated by CRF and negatively regulated by glucocorticoids.

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Introduction

As a member of the corticotropin-releasing factor (CRF) family peptides, urocortin (Ucn 1) was discovered by screening of a rat brain cDNA library with a probe derived from carp urotensin I (Vaughan *et al.* 1995). Human stresscopin (SCP) and stresscopin-related peptide (SRP; Hsu & Hsueh 2001) and mouse Ucn 2 and Ucn 3 (Lewis *et al.* 2001, Reyes *et al.* 2001) corresponding to SRP and SCP respectively were then simultaneously discovered as new members of the CRF family peptides. The actions of these CRF family peptides are mediated by its two receptor subtypes, CRF₁ and CRF₂ receptors (Chen *et al.* 1993, Liaw *et al.* 1996, Kostich *et al.* 1998, Wang *et al.* 2004, Chen *et al.* 2005). The CRF₂ receptor is further divided into two isotypes (α and β) in rodents. Ucn 1 has higher affinities for both the CRF₁ and CRF₂ receptors than for CRF, while Ucn 2 and Ucn 3 are considered endogenous ligands for the CRF₂ receptor, as they show specific affinities for the CRF₂ receptor (Hsu & Hsueh 2001, Reyes *et al.* 2001).

An immunohistochemical study has shown that Ucn 2-like immunoreactivity is present in the posterior pituitary and atria of mice (Hsu & Hsueh 2001), while reverse transcription PCR (RT-PCR) analysis of Ucn 2

mRNA expression has indicated that Ucn 2 is expressed in various human peripheral tissues such as the heart, kidney, spleen, lung, muscle, stomach, testis, thyroid, placenta, adrenal glands, and blood cells (Reyes *et al.* 2001, Tanaka *et al.* 2003). Another recent study, in mice, has shown that Ucn 2 is strongly expressed in skin and skeletal muscle, but weakly expressed in the adrenal glands, stomach, ovary, testis, thymus, brown fat, spleen, and lungs (Chen *et al.* 2004). We have also previously shown that Ucn 2 is distributed in various tissues of rats including the anterior and intermediate pituitary and the adrenal glands, and that Ucn 2-positive cells in the anterior and intermediate pituitary are also positive for β -endorphin, indicating that Ucn 2 is synthesized in proopiomelanocortin (POMC) cells of the pituitary (Yamauchi *et al.* 2005). However, the regulatory mechanisms involved in the synthesis and secretion of Ucn 2 by the pituitary remain unknown. Thus, using primary cultured anterior or intermediate lobe cells of rat pituitary, we examined whether mRNA expression and secretion of pituitary Ucn 2 is affected by CRF, vasopressin, dopamine and glucocorticoids, which influence the synthesis and secretion of POMC-derived peptides.

Materials and Methods

Reagents

DMEM/F12 media, dopamine, and dexamethasone were purchased from Sigma-Aldrich (St Louis, MO, USA). CRF and Ucn 2 were obtained from the Peptide Institute (Osaka, Japan) and the Yanaihara Institute (Shizuoka, Japan) respectively.

Animals

Male Wistar rats weighing 160 g were used for all experiments. All experimental procedures were conducted in accordance with the guidelines on the use and care of laboratory animals approved by the Local Animal Ethics Committee of Nippon Medical School, Japan.

Primary culture of pituitary cells

Thirty rats were killed by decapitation and their pituitary glands were removed under sterile conditions. The anterior and intermediate/posterior pituitaries were separately collected, and mechano-enzymatically dispersed as described previously with modifications. Briefly, pituitaries were minced in a 1:1 mixture of DMEM and Ham's nutrient mix F-12 containing 10% FCS, penicillin and streptomycin (DMEM/F12; Sigma-Aldrich). Samples were then washed twice in PBS and then incubated at room temperature in the PBS containing 0.047 g/l MgCl₂, 0.1 g/l CaCl₂, and 0.01% dispase (Godoshusei, Tokyo, Japan) with constant stirring for 30 min. A 1 ml aliquot of cell suspension containing 1.0×10⁴ cells in a DMEM/F12 was placed in each well of 24-well plates. The anterior and intermediate pituitary cells obtained from 30 rats were cultured in 72 and 18 wells respectively. The cells were subsequently allowed to attach to the surface of the wells in a humidified 95% air–5% CO₂ incubator for 4 days. On the day of the experiment, the culture medium was changed. The cells were incubated for 4 h with the prepared samples and the culture medium from each well then collected. After centrifugation to remove debris at 3500 r.p.m. for 10 min at 4 °C, the culture media from each sample was frozen and kept at –20 °C until assay. Adreno-corticotrophic hormone (ACTH) in the culture medium was measured using an ACTH IRMA kit (Mitsubishi Kagaku Iatron, Tokyo, Japan). β-Endorphin was measured using a high sensitivity EIA kit for rat β-endorphin (Peninsula Laboratories, Inc, San Carlos, CA, USA). Cultured anterior or intermediate lobe cells were homogenized in deionized water by a sonicator and the supernatant after centrifugation was assayed with RIA for Ucn 2 to test its parallelism with the standard curve. Monolayer culture of rat anterior and intermediate pituitary cells was performed six times, and 180 rats in total were used.

RIA for Ucn 2

RIA for Ucn 2 was performed as reported previously (Yamauchi *et al.* 2005). Synthetic mouse Ucn 2 was

Table 1 Primer sequences of the studied genes

Genes	Primers	Length (bp)	Accession number (Genbank)
Ucn 2	Sense: 5'-TGGGCACCTGGTGTATTATGG-3'	374	AY044835
	Antisense: 5'-CCAGAACTCTCATCAGGGTCAC-3'		
	Competitor sense: 5'-TGGGCACCTGGTGTATTATGGTGGCTCTCATGTTGGATAGGGGTC-3'	202	
	Competitor antisense: 5'-CCAGAACTCTCATCAGGGTCCATCCAGGGAGAGTATG-3'		
POMC	Sense: 5'-GAGATTCTGCTACAGTCGCTCAGG-3'	219	AF510391
	Antisense: 5'-CGGGGATTTTCAGTCAAGGG-3'		
	Competitor sense: 5'-GAGATTCTGCTACAGTCGCTCAGGGCTCTCTTCAGACCTCCATAGAC-3'	164	
	Competitor antisense: 5'-CGGGGATTTTCAGTCAAGGGAGGGGTTCATCTCCGTTG-3'		
β-actin	Sense: 5'-TCATGAAGTGTGACGTTGACATCCGT-3'	285	BC063166.1
	Antisense: 5'-CCTAGAACGATTGCCGGTGCACCCGATG-3'		

Figure 1 A standard curve of RIA for Ucn 2 and dilution curves of culture media and extracts of anterior and intermediate lobe cells. AP high K and IP high K indicate culture media in which anterior and intermediate pituitary lobe cells were incubated with 50 mM KCl respectively for 4 h. AP and IP cells indicate the extracts of cultured anterior and intermediate pituitary lobe cells respectively.

Figure 2 Ucn 2 is secreted from anterior and intermediate pituitary cells. Anterior and intermediate pituitary cells were treated with 50 mM KCl for 4 h. After incubation, culture media were collected and assayed for Ucn 2 using RIA with a specific antibody against mouse Ucn 2. Ucn 2 concentrations in culture media are shown as means $\pm$ S.E.M. The number of wells for each treatment was 12. * $P < 0.05$, compared with control.

iodinated using the chloramine-T method and purified on a column of Sephadex G-50 as previously described (Shibasaki *et al.* 1984). PBS (pH 7.5) containing 0.01% NP-40 (Nacalai Inc., Kyoto, Japan), 5 mM EDTA-Na, and 0.02% sodium azide was used for RIA. Synthetic mouse Ucn 2 or sample was incubated with anti-Ucn 2 antiserum (SR-6) in 5 ml plastic tubes for 24 h at 4 °C. ^{125} I-Labeled Ucn 2 (5000 c.p.m.) was then added to each tube and incubated for another 24 h at 4 °C. Goat anti-rabbit γ -globulin was used to separate tracer bound to antiserum from free tracer. The anti-Ucn 2 serum was used for RIA at a final concentration of 1/350 000 to yield a maximum binding of approximately 30%. The cross-reactivity of the antiserum with rat CRF was 0.0% at ED_{50} (Yamauchi *et al.* 2005).

Figure 3 CRF induces Ucn 2 secretion from anterior pituitary cells. Anterior pituitary cells were treated with CRF or vasopressin at concentrations ranging from 1 to 100 nM for 4 h. After incubation, culture media were collected and assayed for Ucn 2 using RIA with a specific antibody against mouse Ucn 2 (A) and ACTH using an ACTH IRMA kit (B). The concentrations of Ucn 2 (A) and ACTH (B) are shown as means $\pm$ S.E.M. The numbers of wells for each treatment in Ucn 2 and ACTH assay were 24 and 12 respectively. * $P < 0.05$, compared with control.

Figure 4 CRF induces Ucn 2 mRNA expression in anterior pituitary cells. Anterior pituitary cells were treated with CRF or vasopressin at concentrations ranging from 1 to 100 nM for 4 h. Total RNA was extracted from cells and assayed for Ucn 2 (A) and POMC (B) mRNA expression levels using competitive RT-PCR. The data shown are representatives of each competitive RT-PCR experiment. Signals were measured using NIH Image and data represent means $\pm$ S.E.M. The number of wells for each treatment was 24. * $P < 0.05$, compared with control.

RT-PCR

Total RNA was extracted from cells using Isogen according to the manufacturer's instructions (Takara, Shiga, Japan). To avoid false positive results caused by DNA contamination, a DNase treatment for 60 min at 37 °C using RNase-free DNase (Takara) was performed. First, strand cDNA was synthesized using 1 µg denatured total RNA under conditions of 42 °C for 30 min, 99 °C for 5 min, and 5 °C for 5 min using the RT-PCR kit (Takara). PCR was carried out under conditions of denaturation at 94 °C for 10 s, annealing at 50 °C for 5 s, and extension at 72 °C for 60 s for 30 cycles, using specific primers for *Ucn 2* and POMC (Table 1). After amplification, the PCR products were subjected to 3% agarose gel electrophoresis, stained with 0.5 µg/ml ethidium bromide and then visualized under u.v. illumination. All PCR-amplified DNAs were sequenced for purposes of confirmation.

Competitor construction

Homologous competitive internal standards that shared the same primer-binding sites, but contained a shortened internal sequence with respect to the endogenous target RNA for *Ucn 2* or POMC, were prepared as follows. The products resulting from PCR were subcloned into the pGEM-T vector (Promega) and sequenced. The cloned pGEM-T vector was linearized by Nco I restriction enzyme digestion and transcribed into a cRNA template by SP6 RNA Polymerase (Promega). The DNA template was removed by RNase-free DNase I digestion at 37 °C for 30 min after transcription and the cRNA product then quantified and used as an internal standard in RT-PCR for *Ucn 2* or POMC.

Competitive RT-PCR

After DNase treatment, the amount of mRNA present in the samples was normalized using β -actin primers (Table 1) as an internal reference standard under the conditions of 94 °C for 10 s, 60 °C for 5 s and 72 °C for 60 s for 30 cycles. The cycle number was determined at the point where PCR amplification is in a linear range by testing each reaction at different dilutions of cDNA for various amplification cycles. To test for possible genomic DNA contamination, either the RT enzyme or RNA was omitted from the reaction tube. To confirm, the RNA competitor was not contaminated with DNA, RT-PCR was performed using only RNA competitor (10^9 copies) at the maximum amount. The reaction mixture and RNA competitor were added to each tube. Reverse transcription reaction was then carried out under conditions of 42 °C for 30 min, 99 °C 5 min and 5 °C 5 min. Next, the PCR mixture was dispensed into each tube which contains RT reactant. PCR was carried out under the following conditions: denaturation at 94 °C for 10 s, annealing at 60 °C for 5 s, and primer extension at 72 °C for 60 s for 40 cycles. The PCR products were separated on 3% agarose gel, and visualized with ethidium bromide. The

intensities of the bands of the PCR products of *Ucn 2* and POMC were quantified using NIH image software. The ratio of endogenous product to each competitor area was plotted as a function of the competitor concentration added to each PCR. The concentrations of *Ucn 2* and POMC mRNA were determined at the point where the ratio of endogenous production to competitor area of each gene was 1.0 (the equivalence point).

Statistical analysis

Statistical analyses were performed using ANOVA followed by Fisher's Protected Least Significant Difference (PLSD) using StatView 4.5 (Abacus Concepts, Inc. Berkeley, CA, USA). Cell culture in same experimental protocol was repeated two to four times and data were combined and

Figure 5 Dexamethasone suppresses *Ucn 2* secretion from anterior pituitary cells. Anterior pituitary cells were treated with dexamethasone at concentrations ranging from 10 pM to 100 nM for 4 h. Culture media were assayed for *Ucn 2* using RIA with a specific antibody against mouse *Ucn 2* (A) and ACTH using an ACTH IRMA kit (B). The concentrations of *Ucn 2* (A) and ACTH (B) are shown as means $\pm$ s.e.m. The number of wells for each treatment was 24. * $P < 0.05$, compared with control.

analyzed. For competitive RT-PCR data, all results were combined for the statistical analysis and expressed as percent of controls. Statistical significance was established at the $P<0.05$ level.

Results

Ucn 2 secretion from anterior and intermediate pituitary cells

Dilution curves of culture medium in which anterior or intermediate pituitary cells were incubated with 50 mM KCl and the extract of cultured anterior or intermediate lobe cells were almost parallel with the standard curve of Ucn 2 (Fig. 1). The basal concentrations of Ucn 2 in the culture medium of the anterior and intermediate pituitary cells were 24.8 ± 1.6 and 23.6 ± 1.8 pg/ml per 10^4 cells per 4 h respectively. When cells were depolarized with 50 mM KCl for 4 h, the concentrations of Ucn 2 in the culture medium of the anterior and the intermediate pituitary were significantly increased to 43.1 ± 2.0 and 38.6 ± 2.3 pg/ml per 10^4 cells respectively (Fig. 2).

Effects of CRF and vasopressin on mRNA expression and secretion of Ucn 2 in anterior pituitary cells

The secretion of Ucn 2 was increased by CRF at concentrations of 10 and 100 nM, while it was not affected by vasopressin (Fig. 3A). Under the same conditions, both CRF and vasopressin significantly increased ACTH secretion at concentrations of 1, 10 and 100 nM (Fig. 3B). The expression levels of Ucn 2 mRNA as well as POMC mRNA were significantly increased by CRF at concentrations of 10 and 100 nM, while they were not affected by vasopressin even at a concentration of 100 nM (Fig. 4A and B).

Suppressive effect of dexamethasone on mRNA expression and secretion of Ucn 2 in anterior pituitary cells

Dexamethasone significantly suppressed Ucn 2 secretion at concentrations of 10 and 100 nM whereas it did not induce any significant change in ACTH secretion (Fig. 5). Dexamethasone significantly inhibited Ucn 2 mRNA expression levels at a concentration of 100 nM while it also inhibited POMC mRNA expression levels at concentrations ranging from 0.1 to 100 nM (Fig. 6).

Figure 6 Dexamethasone suppresses Ucn 2 mRNA expression in anterior pituitary cells. Anterior pituitary cells were treated with dexamethasone at concentrations ranging from 0.1 nM to 100 nM for 4 h. Total RNA was extracted from cells and assayed for Ucn 2 (A) and POMC (B) mRNA expression levels using competitive RT-PCR. The data shown are representatives of each competitive RT-PCR experiment. Signals were measured using NIH Image and data represent means $\pm$ S.E.M. The number of wells for each treatment was 24. * $P<0.05$, compared with control.

Figure 7 CRF induces the secretion and mRNA expression of Ucn 2 in intermediate pituitary cells. Intermediate pituitary cells were treated with 10 nM CRF for 4 h. Culture media were assayed for Ucn 2 and β-endorphin using RIA and a rat β-endorphin EIA kit respectively (A and B). The concentrations of Ucn 2 and β-endorphin are shown as means ± S.E.M. The number of wells for each treatment in Ucn 2 and β-endorphin assays were 16 and 12 respectively. Total RNA was extracted from cells and assayed for Ucn 2 and POMC mRNA expression levels using competitive RT-PCR (C). The data shown are representatives of each competitive RT-PCR experiment. Signals were measured using NIH Image and data represent means ± S.E.M. The number of wells for each treatment was 12. * $P < 0.05$, compared with control.

Effects of CRF and dopamine on secretion and mRNA expression of Ucn 2 in intermediate pituitary cells

CRF significantly increased the secretion of Ucn 2 as well as β-endorphin and the expression levels of Ucn 2 and POMC mRNA at a concentration of 10 nM (Fig. 7). Although dopamine inhibited β-endorphin secretion at concentrations of 10 and 100 nM, it did not affect the secretion and mRNA expression levels of Ucn 2 or the expression levels of POMC mRNA at the same concentrations (Fig. 8).

Discussion

We have previously reported that Ucn 2 is biosynthesized by POMC cells in the anterior and intermediate lobe of rat pituitary (Yamauchi *et al.* 2005). The results of the present study indicate that Ucn 2 is secreted from both anterior and intermediate lobe cells of rat pituitary. In addition, we have shown that the mRNA expression level and secretion of Ucn 2 in anterior lobe cells is stimulated by CRF but not by vasopressin. We also found that CRF stimulates the

expression level of POMC mRNA and secretion of ACTH in anterior lobe cells, while vasopressin stimulates the secretion of ACTH but not the expression of POMC mRNA in accordance with previous reports (Giguere & Labrie 1982, Aguilera *et al.* 1983). The adenylate cyclase–protein kinase A (PKA) pathway mediates CRF-induced secretion of POMC-derived peptides, while AVP increases the secretion of POMC-derived peptides via the phospholipase C (PLC)–protein kinase C (PKC) pathway (Mason *et al.* 2002), indicating the intracellular signaling cascade under CRF is different from that under vasopressin. It therefore seems that the secretion of Ucn 2 is dependent on the adenylate cyclase–PKA pathway, while that of POMC-derived peptides is dependent on either the adenylate cyclase–PKA or PLC–PKC pathways.

It is well known that both the secretion of POMC-derived peptides and expression level of POMC mRNA in the anterior pituitary POMC cells are suppressed by glucocorticoids (Suda *et al.* 1988, Levin *et al.* 1993, Wardlaw *et al.* 1998). The present study has shown that dexamethasone inhibits the secretion of Ucn 2 from the anterior pituitary lobe cells. The secretion of ACTH tended to be lower in the presence of dexamethasone

Figure 8 Dopamine does not affect the secretion or mRNA expression of Ucn 2 from intermediate pituitary cells. Intermediate pituitary cells were treated with dopamine for 4 h. After incubation, culture media were collected and assayed for Ucn 2 (A) and β -endorphin (B) using RIA and a rat β -endorphin EIA kit respectively. The concentrations of Ucn 2 and β -endorphin are shown as means $\pm$ S.E.M. Total RNA was extracted from cells and assayed for Ucn 2 (C) and POMC (D) mRNA expression using competitive RT-PCR. The data shown are representatives of each competitive RT-PCR experiment. Signals were measured using NIH Image and data represent means $\pm$ S.E.M. The number of wells for each treatment was 12. * $P < 0.05$, compared with control.

although the decrease was not statistically significant, in accordance with previous reports showing that dexamethasone does not affect basal secretion of ACTH although it inhibits the secretion of ACTH stimulated by CRF in cultured anterior lobe cells of rat pituitary and AtT20/D16v cells (Familar & Funder 1989, Horiba *et al.* 1993). In the present study, dexamethasone also inhibited the expression levels of both Ucn 2 and POMC mRNA. The actions of corticosteroids are mediated through binding to two distinct intracellular transcription factors, glucocorticoid receptor (GR) and mineralocorticoid receptor (MR). Ligand-activated GR and MR bind to a consensus simple hormone response element in the promoter region of target genes, and activate or inhibit transcription. In the pituitary, the cerebral cortex, hypothalamic paraventricular nucleus, and the hippocampus of rodents are considered to be GR-rich tissues. Although the levels of GR expression differ among reports, several studies have shown GR immunoreactivity in almost all pituitary hormone-producing cells in the rat anterior pituitary, in particular in somatotrophs, corticotrophs, and folliculostellate cells (Kononen *et al.* 1993). It has also been demonstrated that the levels of Ucn 2 mRNA expression in the hypothalamus and brain stem of mice tested by RT-PCR are increased by dexamethasone and decreased by adrenalectomy (Chen *et al.* 2003), while a report using *in situ* hybridization has shown no effect of adrenalectomy on the

expression level of Ucn 2 mRNA in the paraventricular nucleus of rat hypothalami (Tanaka *et al.* 2003). Moreover, adrenalectomy significantly increases Ucn 2 mRNA expression in the skin of rats, and the expression is reversed to normal levels after corticosterone replacement (Chen *et al.* 2004), suggesting that Ucn 2 mRNA expression in the skin is negatively regulated by corticosterone. The same report also detected no effects of adrenalectomy on the level of Ucn 2 mRNA in the skeletal muscle. Several glucocorticoid response element (GRE) consensus sites are located in the 5'-untranslated region of mouse Ucn 2 sequence (Chen *et al.* 2003). Therefore, the effects of glucocorticoids on Ucn 2 mRNA transcription most likely differ among tissues, and Ucn 2 mRNA transcription may be negatively regulated by GR in the anterior pituitary.

The present study has shown that the secretion of β -endorphin and mRNA expression level of POMC in intermediate pituitary lobe cells are stimulated by CRF in accordance with previous reports (Loeffler *et al.* 1986, Saland *et al.* 1991), and that the secretion and mRNA expression level of Ucn 2 in intermediate lobe cells were also stimulated by CRF. The secretion of β -endorphin and the expression of POMC mRNA in intermediate lobe cells are known to be inhibited by dopamine through highly expressed D_2 receptor (Cote *et al.* 1986, Chronwall *et al.* 1988, Loeffler *et al.* 1988, Sands *et al.* 1997). However, the present study showed that

dopamine did not affect either the secretion or the mRNA expression of Ucn 2 in intermediate lobe cells, although it significantly inhibited the secretion but not mRNA expression of β -endorphin. The reason why dopamine did not inhibit the expression level of POMC mRNA may be the shorter incubation of these cells with dopamine, as intermediate lobe cells were treated with dopamine for 48 h in the previous study that detected the suppression of POMC mRNA expression (Loeffler *et al.* 1988). Since dopamine inhibited the secretion of β -endorphin but not Ucn 2, Ucn 2 and β -endorphin appear not to be contained in the same secretory vesicles.

The role of pituitary Ucn 2 is unknown. *In situ* hybridization analyses and RNase protection assays have shown that CRF₂ receptor mRNA is expressed in rat anterior pituitary cells (Chalmers *et al.* 1995, Kageyama *et al.* 2000). In addition, neither SRP nor Ucn 2 affects ACTH secretion from cultured rat anterior pituitary cells *in vitro* or *in vivo* (Hsu & Hsueh 2001, Pellemounter *et al.* 2004). We also found no significant effect of Ucn 2 on ACTH secretion in the cultured rat anterior pituitary cells (data not shown). These findings suggest that pituitary Ucn 2 may act on some pituitary cells other than POMC cells through a paracrine pathway and play some role in the regulatory mechanism of expression/secretion of pituitary hormones.

In summary, the present study demonstrates that the mRNA expression level and secretion of Ucn 2 are increased by CRF in the anterior and intermediate pituitary cells, and that Ucn 2 secretion is suppressed by glucocorticoids in anterior pituitary cells.

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