An integrated map of corticotropin-releasing hormone signaling pathway

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Summary: This study depicts the molecular reactions induced by corticotropin-releasing hormone in the form of a signaling pathway. For this, we have curated all molecular reactions reported from published literature in the context of CRH signaling. This pathway was developed as a part of NetPath, a freely available resource of human signaling pathways developed by our group.

Abbreviations:

CRH - Corticotropin-releasing hormone
CRF - Corticotropin-releasing factor
CRHR - Corticotropin-releasing hormone receptor
PVN - Paraventricular nucleus
GPCR - G-protein coupled receptor
CRHR1 - type 1 Corticotropin-releasing hormone receptor
CRHR2 - type 2 Corticotropin-releasing hormone receptor
CRHBP - Corticotropin-releasing hormone binding protein
POMC - Pro-opiomelanocortin
ACTH - Adrenocorticotropic hormone
PPI - Protein-protein interaction
PTM - Post-translational modification
HPRD - Human Protein Reference Database
SBML - Systems Biology Markup Language
PSI-MI - Proteomics Standards Initiative for Molecular Interaction
BioPAX - Biological Pathway Exchange

Introduction

Corticotropin-releasing hormone (CRH), also known as corticotropin-releasing factor (CRF), is a 41-amino acid neuropeptide, expressed abundantly by CRH neurons present in the paraventricular nucleus (PVN) of the hypothalamus, and other parts of the brain (Aguilera and Liu 2012, Vale et al. 1981). CRH is also expressed in adrenal gland, immune cells, placenta, testis, spleen, gut, thymus and skin (Dautzenberg and Hauger 2002, Aguilera and Liu 2012). The activity of the hypothalamic CRH neurons varies in resting and stress conditions. Circadian variations have an effect on CRH neuron activity. On stressful stimuli, sensory information is either transmitted directly to the PVN or integrated by the limbic system and transmitted to the CRH neurons via complex monoaminergic and peptidergic neural pathways. This is dependent on the nature, intensity and duration of the stressor. Systemic and metabolic stressors including blood loss, pain, immune challenge and hypoglycemia, which require immediate response, utilize monosynaptic pathways, whereas, psychogenic stressors utilize complex multisynaptic pathways. These neural pathways trigger signaling in CRH neurons, increasing CRH gene transcription and rapid CRH secretion (Aguilera and Liu 2012). The actions of CRH are transduced through CRH receptors, which belong to the class II/secretin-like family of the G-protein coupled receptor (GPCR) superfamily (Martin et al. 2005). There are three types of CRH receptors – type 1 (CRHR1), type 2 (CRHR2) and type 3 (CRHR3). Among these, CRHR3 has not been identified in mammals. CRHR1 and CRHR2 are encoded by different genes. CRHR1 mRNA has several splice variants encoding different isoforms – R1α, R1β, R1c, R1d, R1e, R1f, R1g and R1h, predominant of which is CRHR1α (Chen et al. 1993, Ross et al. 1994, Grammatopoulos et al. 1999, Pisarchik and Slominski 2001, Pisarchik and Slominski 2004). CRHR1 is expressed predominantly in the CNS, pituitary, heart (Chen et al. 1993) and also in adrenal gland, ovary, and placenta (Grammatopoulos et al. 1999, Seres et al. 2004, Asakura et al. 1997). The gene encoding the human CRHR2 has three mRNA splice variants which encodes 3 isoforms - R2α, R2β and R2γ and is expressed predominantly in brain and heart (Kostich et al. 1998, Yang et al. 2010). CRH is a high-affinity ligand of CRHR1. It also binds to CRHR2, but with lower affinity (Hsu and Hsueh 2001, Grammatopoulos et al. 1999, Wille et al. 1999). CRH receptors do not have any intrinsic kinase activity and the signal is transduced via the heterotrimeric G-proteins (Freissmuth et al. 1989). Biological activity of CRH is regulated by CRH binding protein (CRHBP), which by binding to the former with high affinity, makes it unavailable for binding to CRH receptors (Potter et al. 1991, Behan et al. 1995, Seasholtz et al. 2002). CRHBP is expressed in high levels in the brain, heart, lungs, and placenta (Potter et al. 1991, Behan et al. 1995, Binder and Nemeroff 2010). CRH exerts its functions through activation of several signaling pathways including adenylate cyclase/protein kinase A (PKA), phospholipase C (PLC)/protein kinase C (PKC), and mitogen-activated protein kinase (MAPK) pathways.

CRH, as a principal mediator of endocrine stress response, activates the HPA axis by binding to the CRHR1 in the anterior pituitary. This, through a cascade of reactions, increases the expression of pro-opiomelanocortin (*POMC*) gene and the release of POMC-derived peptides, adrenocorticotropic hormone (ACTH) and β-endorphin. ACTH, in turn, stimulates the secretion of glucocorticoids from adrenal cortex (Vale et al. 1981). CRH is involved in the etiologies of several stress-related physiological responses and behavioral responses, such as altered blood pressure, increased arousal, enhanced learning, thermogenesis, anxiogenesis, anhendonia,

reduced sleep, psychomotor alterations, decreased appetite and libido (Binder and Nemeroff 2010). CRH, upon binding to hippocampal CRHR1, mediates stress-induced enhancement of fear conditioning and learning. In contrast, CRH by binding to the lateral septal CRHR2 mediates stress-induced anxiety and impairs fear conditioning and learning (Radulovic et al. 1999). Pro-inflammatory and anti-inflammatory responses are exerted through peripheral and central CRH, respectively (Karalis et al. 1991, Friedman and Irwin 2001). Binding of peripheral and central CRH to their receptors were implicated in hemodynamic actions (Yang et al. 2010). Endometrial CRH was found to be involved in stromal cell decidualization during estrus cycle (Zoumakis et al. 2000) and also in implantation of blastocyst (Athanassakis et al. 1999). Placental CRH is involved in the maintenance of pregnancy and onset of labor (McLean et al. 1995). Excess secretion of CRH during severe depression and its association with increased levels of cortisol have been observed (Widerlov et al. 1988). CRH was also reported to be involved in anxiety disorders (Roy-Byrne et al. 1986), and anorexia nervosa (Connan et al. 2007). Decrease in cortical CRH content was observed in Alzheimer's disease (Heilig et al. 1995) and Parkinson's disease (Suemaru et al. 1995).

Diverse aspects of CRH signaling have been well-studied. Owing to its high biological significance, a detailed documentation of all molecular reactions in a centralized resource and depiction of these reactions into a pathway map is desired in the public domain. Therefore, we have curated each pathway reaction reported downstream to CRH-CRHR interaction and submitted a detailed CRH signaling pathway data to the NetPath (http://www.netpath.org) (Kandasamy et al. 2010). Several such ligand-receptor signaling pathways including leptin (Nanjappa et al. 2011), TWEAK (Bhattacharjee et al. 2012) and Prolactin (Radhakrishnan et al. 2012), had been developed by our group and submitted to NetPath. Here, we have described the generation of an integrated pathway map of CRH signaling by manual curation.

Materials and Methods

A survey of scientific literature pertaining to CRH signaling pathway was carried out using PubMed. The search terms that were used include - 'corticotropin-releasing hormone signaling pathway NOT Review' and '(CRH OR CRF OR "corticotropin-releasing hormone") AND (Signaling OR "Signal transduction" OR Pathway OR signal*) NOT Review'. Molecular reactions stimulated by the binding of CRH to CRHR1 and CRHR2 were considered for curation. Reactions induced by the binding of urocortins and non-mammalian CRH-like peptides to CRH receptors were excluded from this study. The molecular reactions that were considered for curation include protein-protein interactions (PPIs), post-translational modifications (PTMs), protein translocation events, activation/inhibition of proteins, and transcriptional regulation of genes and their regulators. The reactions of the signaling pathway were cataloged based on the criteria that have been previously described (Nanjappa et al. 2011, Raju et al. 2011a). PathBuilder (Kandasamy et al. 2009), a pathway data assimilation software previously developed by our group, was used for the annotation of CRH pathway reactions. Specific data pertaining to each of the molecular events has been provided. In addition, information associated with each molecular event including source of the proteins, type of experiment, species of the cells/cell lines used in the experiment, PTM dependence of the event, subcellular localization of the proteins and PubMed identifiers for the research article have been provided. We have also provided reaction comments, with a brief description for each pathway reaction.

We obtained a subset of pathway reactions for CRH signaling by filtering reactions assimilated in NetPath, by applying a set of stringent NetSlim criteria, described previously by our group (Raju et al. 2011b). Based on these confident set of reactions, we developed the pathway map using PathVisio software (van Iersel et al. 2008). The topological arrangement of the molecules in the map was based on the information obtained from, i) inhibitor-based assays; ii) mutation-based assays; iii) knockout studies; iv) canonical pathways; and v) review articles. The CRH pathway map has been provided in the NetSlim resource (http://www.netpath.org/netslim) (Raju et al. 2011b).

Results and Discussion

We have screened over 2,000 research articles until December, 2012 and cataloged molecular reactions from 87 articles. Our cataloging effort of CRH signaling pathway from literature has yielded 73 molecules involved in 20 PPIs, 29 PTMs, 25 activation events, 1 inhibition event and 20 protein translocation events. Of the 20 PPIs, 18 were of direct and 2 were complex type. The upstream enzymes for these 29 PTM events induced by CRH, have not been reported in literature. We could annotate site and residue information for PTMs of 12 proteins. In addition, we have cataloged 25 genes, which are differentially regulated at mRNA level upon CRH signaling in different human cells. The reactions annotated for the CRH pathway have been internally reviewed before being reviewed by a Pathway Authority (SNU, co-author in this manuscript). Pathway data for CRH will be constantly updated in NetPath. Besides, we have submitted 21 reactions to Human Protein Reference Database (HPRD) (Prasad et al. 2009, Goel et al. 2012). CRH signaling pathway is freely available in NetPath (http://www.netpath.org/pathways?path_id=NetPath_129).

These molecular reactions of CRH signaling pathway, cataloged in NetPath, have been graphically depicted using PathVisio (Figure 1). On applying the criteria of NetSlim to the CRH signaling pathway data, 64 molecules involved in 84 reactions were obtained. The NetSlim pathway map generated using this data can be accessed from NetSlim at http://www.netpath.org/netslim/CRH_pathway.html. In the pathway map of CRH signaling, each molecule, represented as node, is linked to its corresponding NetPath molecule. The edge of each reaction is linked to PubMed identifiers. The downstream reactions of the signaling are represented by solid edges for direct reactions and dashed edges for indirect reactions. PPIs, enzyme-catalysis reactions, activation/inhibition reactions and translocation events are depicted in different colors as shown in the legend.

The CRH pathway page in NetPath provides a short description of the pathway, statistics, molecules involved in the signaling reactions and transcriptionally regulated genes of the pathway. Each molecule in the pathway is linked to its NetPath molecule page, which provides information about that molecule and external links to other databases including HPRD (Prasad et al. 2009, Goel et al. 2012), Entrez gene (Maglott et al. 2011), OMIM (Hamosh et al. 2005), and Swiss-Prot (Boeckmann et al. 2003). The curated reactions of CRH pathway can be downloaded from NetPath at http://www.netpath.org/pathways?path_id=NetPath_129. The CRH pathway data from NetPath and NetSlim can be downloaded in different standard data exchange formats including SBML level 2.1 (Hucka et al. 2003), PSI-MI version 2.5 (Hermjakob et al. 2004), and BioPAX level 3.0 (Demir et al. 2010). In addition, gene regulation information is available in tab-delimited and Microsoft Excel formats. We have also submitted CRH signaling pathway to WikiPathways (http://www.wikipathways.org/index.php/Pathway:WP2355).

The mechanisms involved in stress signaling are yet to be fully understood. CRH signaling pathway, being an important mediator of stress responses, has been studied to a considerable extent. A freely available integrated pathway map of CRH signaling will soon become a part of gene set enrichment analysis, which will allow further molecular discoveries in this signaling pathway. These discoveries will pave way for the identification of candidate biomarkers and therapeutic targets in psychiatric and neurological disorders associated with CRH signaling. We

encourage the scientific community to give their valuable suggestions and critical comments to improve the quality and display of information provided in NetPath through http://www.netpath.org/comments

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Conflict of interest

No potential conflicts of interest were declared.

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Figure Legends

Figure 1: Schematic representation of CRH signaling pathway - This map represents the NetPath reactions of CRH signaling pathway. The different types of reactions are distinguished by colors as described in the legend.