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Localization and functional roles of corticotropin-releasing factor receptor type 2 in the cerebellum

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Abstract
The corticotropin-releasing factor (CRF) type 2 receptor has three splice variants α, β, and γ. In the rodent brain only CRF-R2α is present. In the cerebellum, CRF-R2α has two different isoforms: a full-length form (fl) and truncated (tr). Both forms CRF-R2 have a unique cellular distribution. During postnatal cerebellar development, the expression patterns of tr and fl isoforms are changing. This suggests that, CRF and the related peptide urocortin (UCN) could play distinct roles in the immature and adult cerebellum, acting via different receptors subtypes. This review focuses on differences in the distribution of each isoform of CRF-R2 in view of their relationship to CRF and UCN release sites and their possible functional implications. Moreover, it includes novel findings of molecular pathways activating CRF-R2 isoforms through which CRF and UCN excert their specific actions.

Key words: *Purkinje cell, CRF, UCN, CRF-receptors, parallel fibre*

CRF receptors structure and characterization

In the cerebellum, corticotropin-releasing factor (CRF) and related peptide urocortin (UCN), act via two highly homologous G protein-coupled receptors – corticotropin-releasing factor receptors (CRF-R) 1 and 2 (1,2), that are involved in Purkinje cell dendritic growth and differentiation (3,4). UCN has a greater affinity for CRF-R2 than CRF (5,6); both peptides have similar affinities for CRF-R1. CRF-R1 and CRF-R2 receptors stimulate the accumulation of cAMP in response to CRF and/or UCN. CRF and UCN, although acting via the same receptors, are capable of evoking different responses within a single cell. It is hypothesized that the presence of one or more receptor forms in one cell for the same peptide will provide a molecular basis for differential processing (4).

In general, CRF-R1 is abundantly expressed in many regions of the brain including the neocortex, cerebellar cortex, septal zone and amygdala (reviewed in (7,8)). Concerning the distribution of CRF-R2 the evidence is not straightforward. CRF-R2, a 397–438-amino-acid protein, has three splice variants α, β, and γ (Table I) (9,10). The splice variants differ in their N-terminal sequence as well as in their functions and distribution (Table I). CRF-R2γ has been detected only in humans (11), while α and β have been found in rodents as well (9,12). In rodents, CRF-R2β is present in peripheral tissues, while CRF-R2α is localized mainly in the brain (9,10). In contrast to rodents, in man CRF-R2α is found in brain and in peripheral tissues, whereas CRF-R2β and CRF-R2γ occur almost exclusively in the brain (11,13).

CRF-R2α genetics and cerebellar localization

Reports on the presence of the CRF-R2 (α) in the cerebellum are controversial. Van Pett et al. (14) reported the complete absence of CRF-R2 expression in the cerebellar cortex. However, by using different technological approaches, including immunolabelling, western blots and PCR, it has been found that CRF-R2 is widely expressed in the cerebellum (2,8,15–18). It has been shown that two different isoforms of CRF-R2 are present (16–18): one isoform being a full-length form (fl) and the other truncated (tr) (Figure 1; Table II). The forms of CRF-R2 are produced through alternate splicing (Figure 1) (19,20) the CRF-R2 tr isoform encodes a

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Table I. CRF-R2 receptors and their localization in human and rodent tissue.

	Peripheral tissues	Brain
Human	CRF-R2α	CRF-R2α CRF-R2β CRF-R2γ
Rodent	CRF-R2β	CRF-R2 α

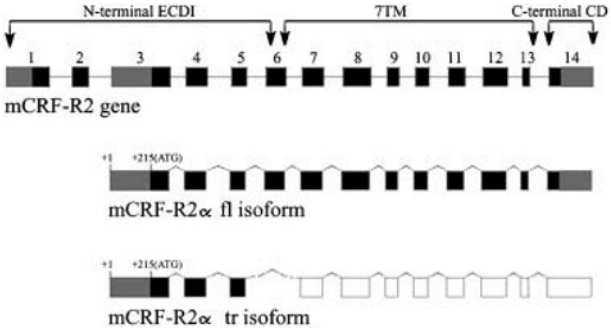

Figure 1. Schematic representation of the rodent CRF-R2 gene. The top line shows the structure of the mCRF-R2 gene. Two functional isoforms of mCRF-R2α: full-length (middle line) and truncated isoform (lower line). Exons coding for the N-terminal ECD, the seven transmembrane domain (7TM) and the C-terminal cytoplasmic domain (CD) are shown. 5′ and 3′-untranslated regions are indicated by hatched boxes, black boxes represent coding regions, and white boxes represent exons downstream to the stop codon (19,21).

236-amino-acid truncated protein, that is the major alternatively spliced isoform of CRF-R2α mRNA transcripts (Figure 1) (21). Possibly, one explanation for the existence of CRF-R2 tr isoform is that it arises as the result of abnormal posttranscriptional processing (splicing errors) (20). The tr-isoform includes three transmembrane domains and a fragment of the fourth transmembrane domain of fl CRF-R2. The remaining membrane loops, as well as, the C-terminus are absent in the tr-isoform (21–23). The molecular weight of the tr-isoform is approximately 22 kDa, that of the fl one is 65 kDa (17,18).

In general, CRF-R2 expression is confined to subcortical structures (1). It has been shown, using an antibody which recognizes both isoforms CRF-R2 (fl and tr), that R2 is expressed in cerebellar climbing fibres from postnatal day (PD) 3-4 until adulthood (8) and in Purkinje cell somata from PD0 onwards (15). During later developmental stages (PD9-15) CRF-R2 was evident in dendritic spines contacted by parallel fibres (8). In addition, during the second postnatal week CRF-R2 was observed in the initial axonal segment of Golgi cells as well as in granule cells (15). In the adult cerebellum, CRF-R2 is localized predominantly in basal regions of the soma and in axon hillocks of Purkinje cells (8). There is a marked increase, during the postnatal development in the overall expression of CRF-R2 (8).

The cellular localization of the CRF-R2 truncated isoform

Each isoform of CRF-R2 has a unique cellular and subcellular distribution in addition to a characteristic developmental expression (Table II). The distribution of the tr and fl isoforms of CRF-R2 has been described in the rodent cerebellum (16–18). In mice, the tr-isoform was observed in the basal region of substantial numbers of Purkinje cell bodies and their initial axonal segments, although not all of them expressed the tr-isoform (Figure 2, Table II). In addition, CRF-R2 tr was observed in the initial segments of Golgi cells and distributed over the molecular layer, CRF-R2 tr was found in parallel fibre terminals (18). In rat, CRF-R2 tr extends into primary dendrites. At the light-microscopic level this was especially abundant and restricted at dendritic branching points. However, there is no evidence at the electron-microscopic level (18). Thus, in the cerebellum, the CRF-R2 tr-isoform has a predominantly axosomal distribution (Table II).

The cellular localization of the CRF-R2 full-length isoform

The full-length isoform of CRF-R2 has a wider distribution than CRF-R2 tr: fewer cerebellar

Table II. CRF-R2 receptor isoforms in cerebellum.

	CRF-R2α	
	tr	fl
Level of expression from PD0 to adult	Increase	Increase
Largest level of expression	Unknown	PD15
Labelling intensity in cerebellum	+	++
Localization in Purkinje cell	Soma (basal region), axon	Soma, axon, dendrites
Labelling intensity in Purkinje cell	+	++
Localization in granular layer	Initial segment Golgi cell	Mossy fibres, soma granular cells, soma Golgi cells
Localization in molecular layer	Parallel fibres	Parallel fibres, soma stellate cells, soma basket cells
Localization in climbing fibres	Absent	Present
Localization in glia	Absent	Bergmann glia, astrocytes

'+' (moderate), '++' (higher) labelling intensity.

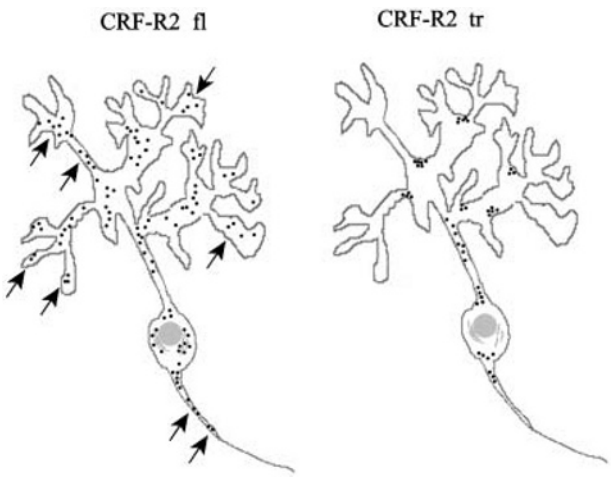

Figure 2. Schematic representation the labelling of CRF-R2 fl (left) and tr (right) isoforms in Purkinje cells. The dots indicate the distribution of the two isoforms, arrows indicate the difference of the labelling between fl and tr isoforms.

neurones are immunopositive for tr isoform than for fl (Table II). This difference was evident as early as the first week of postnatal development in the rough endoplasmic reticulum and the Golgi apparatus of Purkinje cells as well as in climbing fibre terminals ending on somata of Purkinje cells and in mossy fibre rosettes (17). In the next two weeks, the CRF-R2 fl isoforms could be observed in Purkinje cell bodies, in dendrites, axon hillocks and distributed in the molecular and granular layer. In Purkinje cells, labelling intensity for fl isoforms of CRF-R2 is higher than for tr isoform (Figure 2, Table II, (16–18)). The CRF-R2-fl isoform is intensely expressed in the glutamatergic system: granule cells, parallel fibres and mossy fibres rosettes at PD 15 (17). In the adult, CRF-R2 has been observed in most cerebellar neurones: basket, Golgi, stellate and Purkinje cells as well as in climbing and parallel fibres. CRF-R2-fl has also been observed in granule cells, but its presence weakens in the second postnatal week (17). Thus, the first appearance of CRF-R2 fl-isoform in non-Purkinje cells begins between PD 12–15. This suggests that the fl-receptor may be required for late steps of cell migration/positioning and axonal guidance/stabilization of afferent synaptic contacts. Other important changes of fl-isoform, from PD 15 on, is the fl-expression in the shift to the postsynaptic zone of contacts formed between climbing fibres and dendritic shafts of Purkinje cells. Thus, the full length isoform is involved in the programmed regulation of the outgrowth and maturation of Purkinje cell dendrites (17). Finally, CRF-R2 fl is expressed in Bergmann glial cells as well as in astrocytes (16).

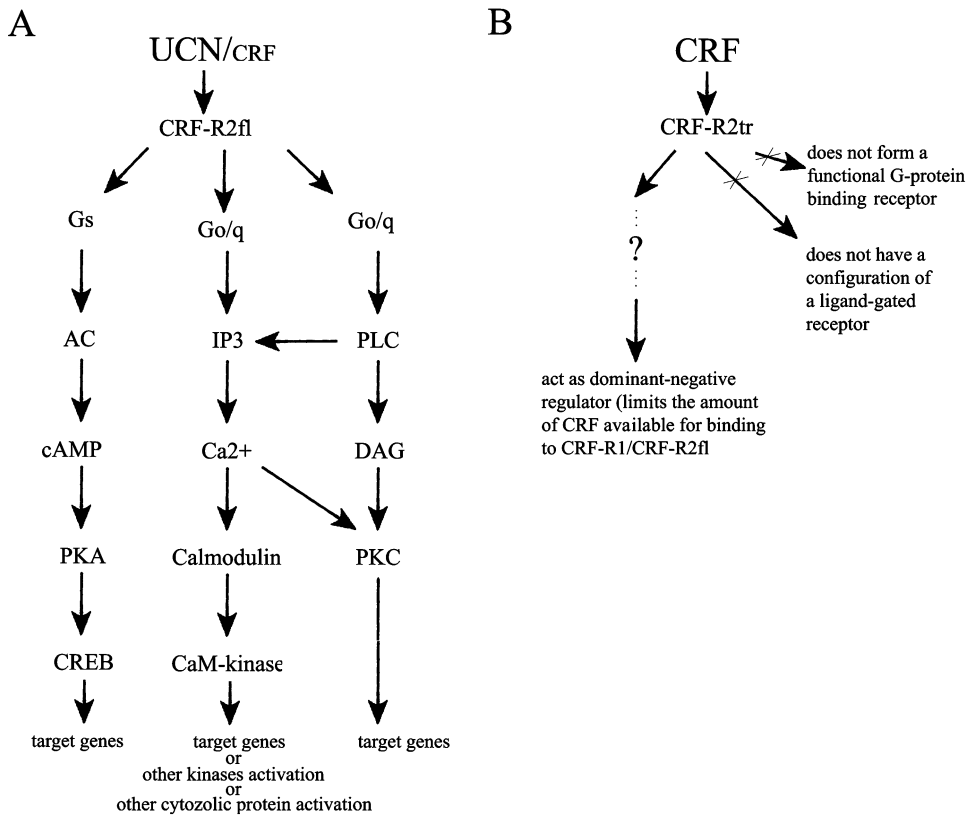

Figure 3. Summary of CRF-R2 specificity and activation. (A) UCN or CRF (with lower affinity) binds to CRF-R2 full-length form receptor to activated PKA or/and PKC-mediated signaling pathway (7,18,24). (B) CRF-R2 truncated isoform binds CRF only and does not act as a ligand-gate receptor or as a G-protein receptor (16,21,23).

Functional roles of CRF-R2 isoforms

The pattern of immunostaining in the cerebellum for tr and fl isoforms is distinct (Table II); the CRF-R2 fl isoform is characterized by numerous profiles that contain more intense immunoreactivity than in the case of the tr isoform. Thus, the change of the expression patterns of tr and fl isoforms of CRF-R2 during postnatal development suggests that CRF and UCN could play distinct roles in the immature and adult cerebellum, acting via different isoforms of CRF-R2 (Figure 3).

The precise role of the truncated receptor has not been defined yet. CRF-R2 tr does not bind UCN and savagine, hence the ligand of this receptor is exclusively CRF (21,23). Furthermore, the truncated isoform does not increase cAMP-level (Figure 3), suggesting that it uses different second messenger pathways and/or subserves a unique function (21). The truncated isoform of CRF-R2 most likely is involved in binding CRF and the presynaptic localization will allow other members of the CRF family of peptides to bind to receptors (full-length isoform and/or CRF-R1) and thus, modulate neuronal activity (Figure 3) (15,17). It could also be involved in the development of extracerebellar afferents and/or be a dominant-negative regulator for CRF (18).

In the case of full-length isoform, receptors stimulate the accumulation of cAMP (Figure 3), the distribution is directly correlated with the developmental stages of the cerebellum, in particular with Purkinje cells and their afferent inputs (17). A strong expression of the full-length form CRF-R2 (not a truncated isoform) in the glutamatergic systems like granule cells, parallel fibres and mossy fibres rosettes, during the end of the second or beginning of the third week of postnatal cerebellar development (mostly at this stage), indicates that the full-length isoform could be involved in the process of the elimination of supernumerary innervation of Purkinje cells by climbing fibres (17).

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