CHAPTER 3

The localisation of urocortin in the adult rat cerebellum: A light and electron microscopic study

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ABSTRACT

Light and electron microscopic immunocytochemistry was used to identify the cellular and subcellular localisation of urocortin in the adult rat cerebellum. Urocortin immunoreactivity (UCN-ir) was visualised throughout the cerebellum, yet predominated in the posterior vermal lobules, especially lobules IX and X, the flocculus, paraflocculus and deep cerebellar nuclei. Cortical immunoreactivity was most evident in the Purkinje cell layer and molecular layer. Reaction product, though sparse, was found in the somata of Purkinje cells, primarily in the region of the Golgi apparatus. Purkinje cell dendritic UCN-ir was compartmentalised, with it being prevalent in proximal regions especially where climbing fibres synapsed, yet absent in distal regions where parallel fibres synapsed. In the Purkinje cell layer, the labelling was also contained in axonal terminals, synapsing directly on Purkinje cell somata. These were identified as axon terminals of basket cells based on their morphology. Terminals of stellate cells in the upper molecular layer also expressed the peptide. Whilst somata of inferior olivary neurons showed intense immunoreactivity, axonal labelling was indistinct, with only the terminals of climbing fibres containing reaction product. UCN-ir in the mossy fibre-parallel fibre system was restricted to mossy fibre rosettes of mainly posterior lobules and the varicose terminals of parallel fibres. Furthermore, labelling also was prevalent in glial perikarya and their sheaths. The current study shows, firstly, that urocortin enjoys a close ligand-receptor symmetry in the cerebellum, probably to a greater degree than CRF since CRF itself is found exclusively in the two major cerebellar afferent systems. Its congregation in excitatory and inhibitory axonal terminals suggests a significant degree of participation in the synaptic milieu, perhaps in the capacity as a neurotransmitter or effecting the release of co-localised neurotransmitters. Finally, its unique distribution in the Purkinje cell dendrite might serve as an anatomical marker of discrete populations of dendritic spines.
INTRODUCTION

Corticotropin releasing factor (CRF) is principally involved in the regulation of the body’s endocrine, autonomic and behavioural response to stress (Koob and Heinrichs, 1999). It plays an important role in the cerebellum, based on its presence and distribution in the embryo (Bishop and King, 1999) and in the adult (Palkovits et al., 1987). CRF-containing projections are particularly prominent in the posterior lobules, especially lobules VIII to X (Overbeck and King, 1999). At a physiological level, Ha et al., (2000) have shown that CRF causes proliferation of cerebellar astrocytes in vitro. Also, Bishop (1990) has shown that CRF augments the activity of excitatory neurotransmitters at the climbing fibre-Purkinje cell synapse and Miyata et al. (1999) have shown that CRF contained in climbing fibres is crucial for the induction of long term depression (LTD), a form of synaptic plasticity proposed as the cellular basis of learning in the cerebellum (Ito. 1984). Taken together, these data suggest that CRF has in the cerebellum, a dual role first in development and later in synaptic plasticity.

The widespread distribution of both CRF receptor 1 (CRF-R1) and CRF receptor 2 (CRF-R2) in the cerebellum (Bishop et al., 2000), coupled with the disparate lobular CRF localisation suggests that other members of the CRF-like peptides could be involved in cerebellar functions. Urocortin is a relatively recent addition to the growing family of CRF-like neuropeptides shown to mediate their effects via both CRF receptors. Urocortin appears to couple preferentially with CRF-R2 leading to speculation that it is the natural ligand of the type two receptor (Vaughan et al., 1995). The localisation of urocortin suggests a role in motor control and sensorimotor integration. The urocortin distribution in the brain is rather restricted, in contrast to CRF, with expression occurring predominantly in the Edinger-Westphal nucleus and the lateral superior olive. Minor sites of expression include the inferior olive, pontine grey, tegmental reticular nucleus, lateral reticular nucleus, and in the red nucleus and nucleus prepositus hypoglossi (for a comprehensive evaluation in rat brain, see Bittencourt et al., 1999). At a physiological level, centrally administered urocortin inhibits food intake to a greater extent than CRF but induces anxiety-like behaviour and locomotor activity to a lesser degree (Spina et al., 1996).

There are conflicting data regarding the precise localisation of urocortin in the cerebellum. Bittencourt et al. (1999) reported weak immunoreactive projections to the molecular, Purkinje cell and granular cell layers of the flocculus, paraflocculus and lingula as well to the deep nuclei of rat cerebellum. Morin et al. (1999), in comparing the distribution of CRF and urocortin in the rat brain made mention only
of CRF “cells” in the cerebellum. Kozicz et al. (1998) also failed to report urocortin cerebellar immunoreactivity in a study of the rat. Using radioimmunoassay, Takahashi et al. (1998), showed a prominent quantity of this peptide in the human cerebellum. Species and methodological differences could account for the incongruencies in the above studies. With a view to elucidating the possible role of urocortin in cerebellar function we provide detailed information on its laminar, lobular and cellular distribution.

**Experimental procedures**

*Immunocytochemistry*

A total of eight black-hooded Lister rats were studied at postnatal day (PD) 40. The day of birth was considered PD 0. Approval to conduct the study was obtained from the Ethics Committee on Animal Experimentation, University of Groningen. All efforts were made to minimise the number of animals used and their suffering. Animals were deeply anaesthetised with sodium pentobarbital (Nembutal®, i.p., 50mg/kg) and perfused transcardially first with a solution containing 2% polyvinyl pyrrolidone (molecular weight 30 000), 0.4% NaNO₃ in 0.1 M phosphate buffer (PB) (pH 7.4) for one minute, and then with 4% paraformaldehyde and 0.2% picric acid in 0.1M PB (pH 7.4) for ten minutes. The brains were removed and stored overnight in the fixative solution at 4°C.

For light microscopy, brains were stored overnight at 4°C in 30% sucrose for cryoprotection and cryo-sectioned (30µm thick) in either the sagittal or transverse plane. Three animals were evaluated in either plane. Immunoreactivity was visualised by the avidin-biotin-peroxidase complex (ABC) method. Briefly, free-floating sections were immersed for two hours in a pre-incubation medium containing 1% normal rabbit serum and 1% bovine serum albumin in 0.1M phosphate buffered saline (PBS) (pH 7.4). The sections were then incubated overnight at 4°C with affinity purified goat anti-urocortin IgG (Santa Cruz Biotechnologies, Santa Cruz, CA), diluted 1:100 with PBS. After washing with PBS, the sections were incubated with biotinylated rabbit anti-goat IgG, diluted 1:200 in PBS, for two hours at room temperature. After further washing, the sections were incubated with ABC (Vectastain Elite, Vector Labs, Burlingame, CA) for one hour at room temperature. Immunoreactivity was visualised by incubation with 5mg 3,3 diaminobenzidine HCl (DAB) and 0.03% hydrogen peroxide in 10 ml of 0.1 M PBS (pH 7.4) for 5 to 10 minutes at room temperature. The reaction was stopped by washing the sections in cold PBS. In some sections, immunostaining was further enhanced according to the gold substituted
silver peroxidase (GSSP) method (van den Pol and Gorcs, 1985).

**Controls**
Sections were processed as above with the omission of the primary antibody or with the addition of a blocking peptide (Santa Cruz Biotechnologies, Santa Cruz, CA), diluted 1:100 with PBS.
The sections were mounted on gelatine-coated slides. Some of the sections were counter stained with cresyl violet for 2 minutes, dehydrated and coverslipped with Depex® mounting medium.

**Evaluation of sections**
Images of sections were projected onto paper using a projection microscope and these were plotted by hand. From these plots, a composite sketch was drawn summarising the labelling pattern. Selected sections were photographed on an Olympus Digital camera, mounted on an Olympus B50 optical microscope. These images were adjusted to enhance the contrast and brightness and saved at 300 dpi.

**Transmission electron microscopy**
Single immunolabelling:
From the cerebella of two animals, 50 µm thick sections were cut on a Vibratome in the sagittal and transverse planes and processed as for light microscopy except, that Triton X-100, 0.025% was added to the primary antibody incubation. Immunoreactivity was enhanced according to the gold substituted silver peroxidase method (van den Pol and Gorcs, 1985). Sections were then osmicated in 1% OsO4, 1.5% potassium hexacyanoferrate in 0.1M cacodylate buffer (pH 7.6) for 15 minutes, dehydrated in a graded series of ethanols and embedded in Epon. Semithin sections (1 µm) were cut on an LKB Ultratome, stained with toluidine blue and used for orientation purposes. Ultrathin sections were then cut, counterstained with uranyl acetate and lead citrate, and examined with a Philips CM 100 transmission electron microscope.

Double immunolabelling:
To prove conclusively that climbing fibre terminals contained urocortin, double immunolabelling with urocortin and CRF (a positive marker for climbing fibres) was performed, according to a modified protocol of Yi et al., (2001). Briefly, sections were placed in phosphate buffer (PB) containing 0.1% sodium borohydrde to inactivate residual aldehyde groups in the tissue sections. To improve reagent penetration, the sections were then treated with PB containing 0.05% Triton X-100.
To prevent non-specific binding of the immunoreagents, sections were incubated in blocking solution, which was PBS, pH 7.4, containing 5% normal goat serum (NGS), 5% BSA, and 0.1% CWFS gelatin. After blocking, sections were incubated in a mixture of rabbit anti-CRF (1:1000) and goat anti-urocortin (1:100) primary antibodies diluted with PBS containing 0.2% acetylated bovine serum albumin (BSA-c), (Aurion, Wageningen, The Netherlands) (PBS/BSA-c, pH 7.4). After washes with PBS/BSA-c, sections were incubated overnight in the first secondary antibody, a conjugate which was ultrasmall gold-conjugated F(ab’)2 (0.8nm) fragments of rabbit anti-goat IgG diluted 1:100 with PBS/BSA-c. After washes, silver enhancement was performed according the manufacturer’s instructions. After the first silver enhancement, sections were washed with PB and then incubated with the second secondary antibody, namely biotinylated goat anti-rabbit IgG, diluted 1:200 in PBS. Immunoreactivity was enhanced according to the GSSP procedure (van den Pol and Gorcs, 1985) and the sections processed further for transmission electron microscopy, in the same manner as for single immunolabelling.

**Antiserum**

The antiserum used in this study was raised against a peptide mapping at the carboxy terminus of urocortin of rat origin (identical to corresponding mouse and human sequences). It reacts with urocortin of mouse, rat and human and does not cross-react with corticotropin-releasing factor.

**RESULTS**

**Regional distribution**

Note that due to variations in the intensity of lobular labelling, all micrographs are of regions from either lobule IX or X, unless stated to the contrary.

Urocortin immunoreactivity (UCN-ir) could be visualised in all vermal lobules, the hemispheres as well as the flocculus and paraflocculus of the cerebellar cortex. Labelling appeared to be constant medio laterally, from vermis to hemispheres. However, a distinct staining gradient was noted in the sagittal plane (Fig. 1). The posterior lobules (especially lobules IX and X), the flocculus, and the paraflocculus exhibited the most abundant labelling, closely followed by lobules VII, VIII and then lobule VI. The anterior lobules appeared to contain appreciably less immunoreactivity (Fig. 2). UCN-ir was also present in the white matter, deep cerebellar nuclei (fastigial, interposed and dentate), and the lateral vestibular nuclei (Fig. 3).
Figure 1. Schematic representation of UCN-ir in the mid-sagittal and transverse planes of the cerebellum, concentrating on the labelling in Purkinje cells. (A) shows the vermal lobular immunoreactivity pattern in the sagittal plane with the labelling predominating in posterior lobules, especially lobules IX and X. Labelling is mainly in the Purkinje cell dendrites (arrow) as well as some somata (arrowhead). (B) shows the labelling pattern of the anterior lobules in the transverse plane with the UCN-ir being somewhat weaker. However intense immunoreactivity could be visualised in the deep cerebellar nuclei as well as the lateral vestibular nucleus. (C) shows the labelling pattern of the posterior lobules in the transverse plane with UCN-ir being particularly prominent in the Purkinje cell dendrites in the vermis. C I & II, crus one and two; DN, dentate nucleus; FL, flocculus; PFd, dorsal region of the paraflocculus; PFv, ventral region of the paraflocculus; FN, fastigial nucleus; IN, interposed nucleus; LS, lobulus simplex; Vn, lateral vestibular nucleus.
Figure 2. Photomicrographs of UCN-ir showing differences in intensity between anterior and posterior lobules. (A) In lobule X, the labelling pattern is intense and concentrated in the Purkinje cell dendrites (arrowheads), with weak localisation in the regions of their somata. (B) An overview of lobule II indicating immunoreactivity of relatively weak intensity, and (C), a higher magnification of the framed area showing the labelling being concentrated around the Purkinje cell somata and puncta in the molecular layer (arrowheads). Note that to observe the relatively weak immunoreactivity in lobule II, the DAB reaction product had to be enhanced according to the GSSP method.
Labelled profiles were evident in all layers of the cerebellar cortex, predominantly in the molecular and Purkinje cell layers. Some Purkinje cells contained sparse reaction product in their perikarya, associated with small vesicles in the region of the Golgi apparatus. Axonal labelling was mainly at terminals synapsing on the deep cerebellar nuclei. Dendritic labelling appeared in the main shafts as well as in the spines of the proximal dendritic domain synapsing with climbing fibres. While the somatic and axonal reaction product was conspicuously associated with spherical vesicles, this was not the case in dendrites, where labelling often occurred in large clumps that are most likely related to tubular elements of endoplasmic reticulum (Fig. 4).

In the Purkinje cell layer, varicosities synapsing directly on somata of Purkinje cells showed UCN-ir. These were characteristic of basket cell terminals. In the upper molecular layer, terminals of stellate cells synapsing directly on Purkinje cell dendritic shafts also contained UCN-ir (Fig. 5).

UCN-ir was evident in the mossy fibre-parallel fibre system. Mossy fibre rosettes, especially those in the posterior lobules, exhibited reaction product. In the molecular
Figure 4. Micrographs showing UCN-ir in the Purkinje cell. (A) a photomicrograph showing UCN-ir in the proximity of Purkinje cell somata and proximal dendrites. Arrowheads point to labelled puncta which could be either Purkinje cell spines, parallel fibre varicosities or climbing fibre varicosities. (B) moderate UCN-ir in a Purkinje cell soma is associated with Golgi-like vesicles (arrowheads). In (C), a labelled Purkinje cell dendritic spine (arrowhead) is shown making an asymmetric synapse with an unlabelled terminal varicosity. In the vicinity, three unlabelled dendritic spines (asterisks) can be observed. G, Golgi apparatus; Pc, Purkinje cell
Figure 5. Basket and stellate cell UCN-ir. (A) shows a basket cell with prominent somatic labelling (arrowheads), located in the vicinity of the Golgi apparatus. (B) a detail of a Purkinje cell soma showing labelling in a profile characteristic of a basket cell terminal. (C) Shows reaction product in the upper molecular layer. In the centre, is a stellate cell terminal containing reaction product (arrow heads), making direct synaptic contact on the shaft of a Purkinje cell dendrite. Reaction product is also contained within the glial sheath, the dendritic shaft itself and within parallel fibres in the vicinity. Note that this micrograph is of material sectioned in the transverse plane. Hence parallel fibres are viewed in the longitudinal plane. Bc, basket cell; Bt, basket cell terminal; Gs, glial sheath; Pd, Purkinje cell dendrite; Pf, parallel fibres; Ps, Purkinje cell soma; St, stellate cell terminal.
layer, labelling was evident in the axons of granule cells, that is, parallel fibres. Whilst some of this reaction product was observed in the shafts, most of it was in the synaptic varicosities of the parallel fibres. The palisades of Bergmann’s glia were also labelled (Fig. 6).

At the light microscopic level, no labelled olivo-cerebellar axonal profiles were observed in either the white matter or cortical layers of the cerebellum. Although, in the inferior olive, dense cytoplasmic labelling was evident. However, at the electron microscopic level, profiles in close association with Purkinje cell dendrites and resembling climbing fibre axonal terminals in the molecular layer contained reaction product. The results of the double immunolabelling with antibodies against CRF and urocortin proved these to be climbing fibres since in the molecular layer, CRF is found exclusively in climbing fibres. CRF immunoreactivity is exhibited in the form

![Figure 6](image_url)

**Figure 6.** UCN-ir in the mossy fibre-parallel fibre system and glial elements. (A) a survey of a labelled mossy fibre rosette showing clustered reaction product (arrowheads). (B) an overview of parallel fibre labelling in the shafts (arrows) and smaller vesicular labelling in pre-synaptic varicosities and terminals (arrowheads). The dendritic spines which make synaptic contact with urocortin positive parallel fibres all are free of reaction product. However, a number of other Purkinje cell dendritic profiles exhibit dense labelling (open arrowheads). Note also that the micrograph is of a section, cut in the transverse plane. Therefore, parallel fibres are viewed in the longitudinal plane. (C) shows a region, cut in the sagittal plane, of the upper molecular layer. Reaction product (arrowheads) is present in parallel fibre varicosities making synaptic contact on Purkinje cell dendritic spines. Note that, similar to (B), spines postsynaptic to parallel fibre terminals are free of reaction product. (D) an overview of a basket cell with the insert being a magnified region of the boxed area, showing a labelled glial sheath as it borders the perikaryon. The labelling is in the form of large clumps that almost seem continuous.
localisation of urocortin

Figure 7. UCN-ir in olivo-cerebellar projection neurons. (A) a photomicrograph of the principle and dorsal accessory olive showing prominent somatic labelling. (B) an electron micrograph of urocortin single immunolabelling, according to the GSSP method, showing UCN-ir in a varicose terminal, making synaptic contacts (arrowheads) on two spines of a proximal Purkinje cell dendrite. The terminal shows all the morphological characteristics of a climbing fibre. (C) shows urocortin and CRF double immunolabelling within a climbing fibre terminal. UCN-ir is exhibited in the form of uniform, enhanced ultrasmall (0.8nm) immunogold particles (arrowheads), whereas CRF immunoreactivity appears as clumpy, irregular silver deposits, characteristic of the GSSP method (arrows). Whilst CRF immunoreactivity was confined to climbing fibres UCN-ir was evident in climbing fibres and Purkinje cell dendritic spines. Dao, dorsal accessory olive; Po, principal olive.

of clumpy, peroxidase reaction product and UCN-ir is represented by uniform, ultrasmall immunogold particles (Fig. 7).

DISCUSSION

The present study is the first to comprehensively detail the distribution of urocortin in the adult rat cerebellum. Using light and electron microscopic immunocytochemistry, we were able to pinpoint the localisation of this neuropeptide both at cellular and sub-cellular levels. The present study shows that urocortin is extensively expressed throughout the adult rat cerebellum, with highest amounts occurring in vermal lobules IX and X, the flocculus, and the paraflocculus. This is in contrast to a previous study (Bittencourt et al., 1999) that showed only labelled fibres in the flocculus, paraflocculus and lingula. The main reason for this discrepancy could be the different antisera used. Another reason is that in other regions of the cerebellum where UCN-ir was somewhat weaker, most of the labelling tended to occur in axon terminals, that are more readily visualised at the electron microscopic level. Our study is, however, consonant with the previous finding of prominent labelling in the
cerebellar nuclei and vestibular nuclei (Bittencourt et al., 1999). Additional sites found to be immunoreactive were the synaptic terminals of the inhibitory basket and stellate cells and the Bergmann’s fibres.

**Antiserum specificity**

One of the most crucial elements of immunohistochemical studies is the specificity of the antiserum that ensures a cogent relationship between the reaction product and the antigen under examination. Despite the relatively pervasive labelling (in comparison to CRF) encountered with the antiserum used in the study, we are convinced of its specificity in reliably binding to urocortin for the following reasons. Firstly, labelling was not evenly distributed across the cerebellum but consistently exhibited a distinct gradient in the posterior to anterior plane. Also, labelling tended to be concentrated in specific axonal terminals, namely those of basket, stellate, granule and Purkinje cells. At the electron microscopic level, the UCN-ir was present within these terminals without any aspecific labelling on other profiles. Finally, when somatic labelling occurred, this was never nuclear, but always cytoplasmic, and occurred in close proximity to the Golgi apparatus.

**Urocortin and CRF receptor localisation**

Although purely an immunocytochemical approach, this study suggests an intimate interaction between urocortin and the CRF receptors, obviating, at least in the cerebellum, the constant issue of ligand-receptors mismatches that has beset investigators since the initial CRF studies (Chalmers et al., 1995, Potter et al., 1994). Urocortin is known to mediate its effects by coupling to both known CRF receptors (Vaughan et al., 1995). In the mouse, Bishop et al. (2000) showed that both receptors are localised on Purkinje cells (their somata, dendrites and initial axonal segments) with CRF-R1 alone being further localised in the radial glia as well as in puncta in the granular layer. CRF-R2 is also localised in basket and Golgi cells and the initial axonal segments of Purkinje cells. Some of the most prominent labelling found in this study occurred in the form of axon terminals synapsing on Purkinje cell somata. These are unlikely to be climbing fibre terminals since climbing fibres do not make synaptic contacts on Purkinje cell somata at such late adult stages. Based on their morphology, they are most likely to be the inhibitory terminals of basket cells since these neurons do indeed form axo-somatic synapses with Purkinje cells in adulthood (Palay and Chan-Palay, 1974). Also, the basket cell somata were found to exhibit prominent somatic labelling. However, the possibility of these being the recurrent collaterals of Purkinje cell axons cannot be entirely excluded due to the close structural resemblance of these terminals and those of basket cell axon terminals (Altman and...
Bayer, 1997). Importantly, however, urocortin-containing varicosities synapsing on the somata of Purkinje cells are to be expected since Bishop et al. (2000) has shown that, at least in the mouse, CRF-R2 is located in this region. Stellate cells also exhibited labelling at their synapses on Purkinje dendrites, though to a lesser degree. Another prominent site of labelling was the synaptic varicosities of parallel fibres which form excitatory synapses on Purkinje cell spines. It is rather perplexing why a single neuropeptide would be localised at both excitatory and inhibitory synapses. However, since CRF receptors are localised to postsynaptic sites (Purkinje cell somata in the case of basket cell axons and Purkinje dendrites in the case of parallel fibres), this receptor system could play an integral part of neuromodulation in the cerebellum. The fact that the reaction product at these synapses was localised in vesicles in appreciable amounts suggests some kind of neurotransmitter capacity (Schwartz et al., 2000).

Another major site of UCN-ir was the Purkinje cell itself. The perikarya were weakly labelled in comparison to axons and dendrites. This compartment probably serves merely as the site of synthesis and packaging of the peptide, which may be rapidly transported to the neurites. Labelling in the dendrites was localised in the main shafts and in some spines. Spines which formed synapses with terminals containing reaction product, i.e. parallel fibre-dendritic synapses, did not contain any labelling. However, spines forming synapses with climbing fibre did indeed contain labelling. The labelling pattern observed in this study suggests a compartmentation of urocortin in the Purkinje cell dendrite. Even at the light microscopic level (Fig. 4 A), UCN-ir never reached the distal domain of the dendritic tree. Rather, it predominated in the proximal domain known to be contacted by climbing fibres. This is in keeping with current evidence that individual Purkinje cell dendrites are compartmentalised into two different synaptic domains due to their contiguous innervation by climbing fibres and parallel fibres (Morando et al., 2001; Bravin et al., 1999; Strata et al., 1997). The fact that in axonal elements, UCN-ir is associated with vesicles, whereas dendritic and glial reaction product is not, enhances the supposition that the peptide may have different functions. Studies investigating urocortin distribution in Purkinje cell dendrites after afferent perturbation such as climbing fibre lesioning by 3-acetyl pyridine (Rossi et al., 1991a, b; Desclin and Colin, 1980) or in hypogranular mice mutant (Crepel and Mariani, 1976; Mariani et al., 1977; Crepel et al., 1980) will shed more light on the way its targeting to the dendritic environment is controlled.

A point of contention was the labelling of climbing fibres. Bittencourt et al. (1999) reported reaction product only in projections to the inferior olive and not the cell
bodies themselves. However, the present study shows substantial amounts of reaction product within cell bodies of inferior olivary neurons. Furthermore, no immunoreactivity was readily visible in their axonal shafts at the light microscopic level in any part of the cerebellum. At the electron microscopic level, however, axonal terminals in the molecular layer fitting the profile of climbing fibres did express reaction product. Moreover, double labelling experiments which co-localised CRF and urocortin proved conclusively that urocortin is contained in the terminals and not in the axons of climbing fibres. Hence, it appears that synthesised urocortin in inferior olivary neurons is rapidly transported to the axonal terminals, precluding the localisation of significant amounts in the fibre tracts. If this is the case, then it implies that the inferior olive processes urocortin differently to CRF, since CRF is clearly localised in fibre tracts (Palkovits et al., 1987). The functional significance of this disparate handling of two different peptides which react essentially on the same receptors system remains to be elucidated.

**Urocortin in cerebellar function**

The processes involved in the development of the cerebellar circuitry eventually manifest in a structure pivotal for the fine-tuning of motor coordination (see Welsh et al., 1995; Wickelgren, 1998) and perhaps higher cognitive functions (Muller et al., 1998). An understanding of these events could provide a convenient paradigm for neuronal learning and brain behaviour relationships. The emerging picture of how urocortin, CRF and their receptors are distributed in the cerebellum encourages one to attempt to delineate the roles played by these two peptides in cerebellar function. Urocortin is localised in the targets of both the mossy fibre and the climbing fibre systems, granule cell axons, Purkinje cells, inhibitory interneurons, and the deep cerebellar nuclei. More specifically, the presynaptic location of urocortin in excitatory and inhibitory axonal terminals and its post-synaptic location in the shafts and spines of Purkinje cell dendrites, suggests an integrative role in the neuronal circuit dynamics of the cerebellar cortex.

The observations that vestibulocerebellar folia, i.e. lobules IX and X, the flocculus, and paraflocculus, appeared to be more heavily labelled than the rest of the cortex, suggests that urocortin might be more intimately involved in the vestibular aspects of cerebellar function. Its prominence in this region of the cerebellum is consonant with that of CRF, suggesting that in the adult cerebellum, CRF-like peptides mainly function in vestibular control. Also, one of the most prominent sites of urocortin localisation in the brain is the Edinger-Westphal nucleus. In the cat, this site provides projections to the anterior and posterior cerebellar cortices and the fastigial and
interposed nuclei, with the flocculus being the main terminal field (Roste and Dietrichs, 1988). However, both CRF receptors are, by and large, evenly distributed throughout the mouse cerebellum (Bishop et al., 2000), intimating that other, yet undiscovered CRF-like peptides might be responsible for the activation of CRF receptors resident in these regions.

In conclusion, the present study shows that urocortin has a specific and prominent expression in the cerebellum, adding to the growing body of evidence that this peptide is involved in motor control. The data shown here on the urocortin distribution may also serve as a guide in prospective physiological studies.

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