Chapter 35
Neuropeptides in the Cerebellum

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Abstract To truly understand cerebellar function, it is essential to address the effect of the numerous peptides present within cerebellar circuits and the role they play in modulating neuronal activity in the cerebellum. To date, at least 22 neuropeptides have been identified in the cerebellum. However, relatively little is conclusively known about the modulatory role of the vast majority of these peptides. The potential role of three peptides is reviewed. Future research should focus on defining transduction pathways activated following binding of these peptides to their G-protein-coupled receptors, defining the function of peptides produced by cerebellar neurons such as Purkinje cells or Golgi cells, and describing how neuropeptides modulate cerebellar nuclear neurons, which represent the output of the cerebellum.

Keywords Cerebellar nuclei • Climbing fiber • Corticotropin releasing factor • Orexin • Calcitonin gene related peptide • Purkinje cell

35.1 Introduction

The existence of neuropeptides in the central nervous system has been known since the 1970s (Hokfelt 1991). Today, at least 100 neuropeptides have been identified (Ito 2009). Neuropeptides are polypeptides that almost exclusively bind to G-protein coupled receptors. Thus, their actions require activation of second messenger pathways, which utilize complex intracellular pathways to alter the response properties of neurons. Neuropeptides are often characterized as neuromodulators.

An overview of neuropeptide synthesis, transport, release and removal may be found in a neuropharmacology textbook. A unique feature of neuropeptide synthesis is that it occurs entirely within the cell bodies of neurons and requires active

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transport to an axon terminal. Further, a single gene may produce several different related neuropeptides in different brain regions. Within the presynaptic terminal, large dense core vesicles that contain neuropeptides tend to be located away from the active zone. The mechanism of peptide release is the same as for vesicles containing amino acids, requiring influx of calcium. However, for peptides to be released, more calcium influx is required than that needed for release of amino acid neurotransmitters. One way to accomplish this by a longer train of action potentials coming down the axon. Whereas a single action potential may result in release of amino acids, a prolonged train may be required to allow greater influx of calcium and subsequent release of peptides into the synaptic cleft. Thus peptide release, is activity dependent.

The method for terminating peptide activity is through endopeptidases and exopeptidases. The concentration of these peptidases is relatively low so neuropeptides may diffuse great distances from the site of their release to remote receptors thus giving them the opportunity to effect larger populations of neurons.

35.2 Neuropeptides in the Cerebellum

Table 35.1 shows several peptides that have been identified in the cerebellum and their localization in diverse components of cerebellar circuitry based on a review by Ito (2009).

Table 35.1 Summary of neuropeptide distribution in different neuronal components in the cerebellum

Purkinje cell Lugaro cell Golgi cell	Climbing fiber	Mossy fiber	Beaded fiber	Receptors on granule cells
Atrial natriuretic peptide	Corticotropin releasing Factor	Corticotropin releasing factor	Angiotensin II	A-melanocyte stimulating hormone
Cerebellin	Insulin-like growth factor 1	Cholecystokinin	Dynorphin	Melanin-concentrating hormone
Motilin	Calcitonin gene related peptide	Calcitonin gene related peptide	Leu-Enkephalin	Neuronal neurotensin
		Leu-Enkephalin		
Galanin	Atrial natriuretic peptide	Met-Enkephalin	Met-Enkephalin	Somatostatin
		Substance P	Orexin	Neuropeptide Y

Several neurons within the cerebellar cortex express different neuropeptides. Neuropeptides also have been found in afferent systems to the cerebellum including climbing fibers, mossy fibers and a beaded plexus of axons. Granule cells express receptors for peptides; the origin is yet to be conclusively determined (Adapted from Ito 2009)

35.3 Role of Peptides in the Cerebellum

One of the major challenges to understanding of the role for peptides in the cerebellum is the fact that their distribution varies between and within species. This chapter will describe some of the potential roles for three peptides in regulating cerebellar circuitry.

35.3.1 Corticotropin Releasing Factor (CRF)

CRF is present in climbing and mossy fiber afferent systems in all mammalian species studied to date (Fig. 35.1a, b). CRF in climbing fibers originates from neurons in the inferior olive whereas CRF in mossy fibers originates from the vestibular complex and the reticular formation (Errico and Barmack 1993; Bishop 1998). Neurophysiological studies have shown that CRF is essential in the generation of long-term depression, a mechanism associated with cerebellar learning (Miyata and Ito 1999). Further, CRF has been shown to increase the firing rate of Purkinje cells (Fig. 35.1c, d) by decreasing the amplitude and duration of the after hyperpolarizing potential (Fox and Gruol 1993) and blocking GABA induced inhibition (Bishop 1990) (Fig. 35.1e).

35.3.2 Orexin

Orexin is present in a beaded plexus of axons that originate from neurons located in the perifornical area and the lateral hypothalamus (Zhang et al. 2013). These axons terminate primarily within the flocculus of the cerebellum (Nisimaru et al. 2013). Zhang et al. (2013) suggested that the orexinergic system participates in motor control and integration of somatic motor and non-somatic (e.g., visceral, emotional) systems. They postulated that a somatic-non-somatic integration was critical for generation of a coordinated behavioral response to changes in the internal and external environment. The primary effect of orexin was on neurons located in the vestibular nuclei and the cerebellar interpositus nucleus (Yu et al. 2010) which represent a peptidergic effect on the output neurons of the cerebellum and closely related vestibular system.

35.3.3 Calcitonin Gene Related Peptide (CGRP)

CGRP is transiently expressed in climbing fibers during rodent development (Morara et al. 1992). The CGRP receptor, which mediates the effect of this peptide, is present on astrocytes during early stages of development and on Purkinje cells at

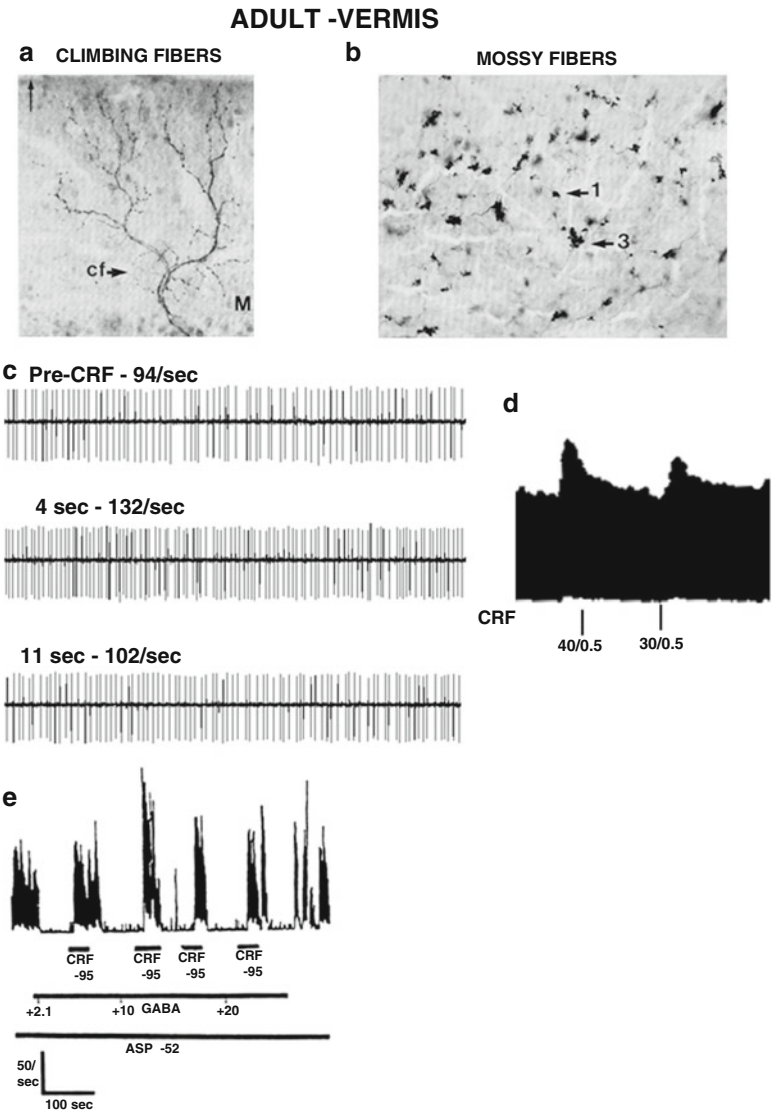

Fig. 35.1 (**a**) CRF-immunolabeled climbing fiber in the opossum cerebellum. (**b**) CRF-immunolabeled mossy fiber in the opossum cerebellum. (**c**) Extracellular recording from a Purkinje cell in the rat's cerebellum. Baseline firing rate was 94 spikes/s. 4 s after application of CRF the firing rate increased to 132 spikes/s. The unit recovered to baseline 11 s after application of CRF. (**d**) Histogram derived from data shown in C. X axis is time. Y axis is spikes/s. The dashed lines indicate application of CRF at the designated pressure and time. Following application of CRF the firing rate of the Purkinje cell increases and remains elevated for a prolonged period of time. The effect is dose dependent. (**e**) Histogram documenting interactions between CRF and GABA. This is from a recording in the rat's cerebellum. Application of aspartate causes the neuron to fire at approximately 60 spikes/s. Application of GABA blocks the aspartate induced excitation. Co-application of CRF during the GABA induced inhibition blocks the suppressive effect of GABA, even if the inhibitory transmitter is applied at higher doses

later stages (Morara et al. 2008). Studies demonstrate that CGRP modulates calcium in astrocytes during development (Morara et al. 2008). During later stages of development, CGRP was shown to stimulate Purkinje cell dendrite growth in culture (D'Antoni et al. 2010), an effect that was dependent on activation of CGRP receptors on astrocytes.

A different pattern of CGRP expression was observed in the cat's cerebellum, compared to the rat's, consistent with unique distributions and functions of peptides in different species. In the cat (Bishop 1992), CGRP was found in mossy fibers in the adult animal. Physiological studies demonstrated that CGRP suppressed spontaneous and excitatory amino acid-induced activity (Bishop 1995). In addition, there was a heterogeneous distribution of CGRP mossy fibers in the cat's cerebellum suggesting that the effect of this peptide is restricted to specific populations of cerebellar neurons (Bishop 1992).

35.4 Conclusion and Future Directions

To truly understand cerebellar function, it is essential to understand the functional role of the numerous peptides present within cerebellar circuits. Likely, many of the peptides expressed in the cerebellum are involved in modulating the activity of Purkinje cells and one, CRF, is essential for generation of long-term depression in the cerebellar cortex. However, to date, relatively little is conclusively known about the modulatory role of the vast majority of these peptides on intact cerebellar circuits. Functionally, it is essential to identify specific second messenger and associated signal transduction pathways and to determine differential roles for peptides during different stages of development and in the adult. Finally, defining the role of neuropeptides in regulating the activity of cerebellar nuclear neurons, which represent the output of the cerebellum, is essential.

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