Chapter I

Introduction

The cerebellum, concerned with co-ordination of somatic motor function and with the maintenance of equilibrium and regulation of muscle tone, surves as an example of the important role sensory integrating mechanisms play in motor function. The integrative influences of the cerebellum can effect activities at all levels of the neuraxis. Afferent cerebellar pathways convey impulses from a wide variety of different receptors, including the organs of special sense. Among these afferent systems, the input from stretch receptors (i.e., muscle spindle and Golgi tendon organ) is especially large. These impulses are conveyed by the spino-cerebellar and cuneo-cerebellar tracts. The principle function of stretch receptors appears to be the unconcious neural control of muscle tone. The cerebellum, which receives the major afferent input from stretch provides part of the neural mechanism that : 1) effects gradual alteration of muscle tensions for proper maintenance of equilibrium and posture, and 2) assures the smooth and orderly sequence of muscular contractions that characterize skilled voluntary movement.

Recent physiological studies of the functional organization of the cerebellar cortex suggest that the cerebellum may function as a kind of computer in the regulation and control of movement (Llinas, 1975). The cerebellum appears to organize and integrate information flowing to it via numerous neural pathways. So that the cerebellum is capable of coordinating movement even in the absence of all information from the periphery of the body. By removing the forebrain and blocking propioceptive sensation in experimental animals ; as long as the cerebellum remained intact locomotion was possible, but it was disrupted when the cerebellum was lacking (Llinas, 1975). The region of the brain where the correlation of anatomy with function has been determined with the greatest success is the cortex, or outer sheath, of the cerebellum. (Llinas, 1975).

Many recent anatomical and neurophysiological studies have greatly clarified the synaptic organization of the cerebellar cortex. In particular, it is generally accepted that information processing in the brain largely involves communication among neurones through release of neurotransmitters at synapses. Theoretically, the brain might do with one excitatory and one inhibitory transmitter Until the 1960 's the amines, acetylcholine, norepinephrine, and

serotonin were the only well - recognized transmitters. (Hökfelt, Johansson, Ljungdahl, Lunberg and Schultzberb, 1980). Then came on appreciation that amino acids such as γ - aminobutyric acid (GABA) , glutamic acid, aspartic acid, and glycine, might serve as transmitters (McLennan, 1963). A dramatic explosion in the number of possible neurotransmitters came with increasing recognition in the past decade that various peptides may be neurotransmitters. Some, such as the opioid peptides enkephalines, neurotensin, and substance P, were first isolated from the brain (Hökfelt et al.1980) Peptides, such as cholecystokinin and vasoactive intestinal polypeptide, were known as intestinal hormones and later recognized as brain constituents. Certain hypothalamic - releasing hormones, pituitary peptides, and blood - derived peptides like angiotensin II and bradykinin, may also be control neurotransmitters. (Snyder, 1980). This was timely because the recent progress in peptide chemistry was ready to promote the subsequent development of the field of peptide neuropharmacology. A considerable amount of accumulated data suggests the transmitter role of certain peptides. Furthermore, the accurence of biologically active peptides with the same neurones containing classical neurotransmitter substances have also been observed (Schultzberb and Höckfelt, 1982). It is important to realize that a transmitter role seems well substantiated for a few

peptides, even crucial experimental evidence is lacking for many others, and additional functions of neuropeptides, as trophic factors or factors involved in long term events (Hökfelt, Johansson, Ljungdahl, Lundberg, and Schultzberg, 1980). Nor does ultrastructural analysis reveals any fine structural features exclusively characteristic of peptide neurones. One feature that is common to several classes of peptide neurone is the large granular vesicle. (Goldsmith, 1977). Although functional evidence suggests that at least some peptides act as neurotransmitters (Otsuka, and Takahashi, 1977), the method of replenishment of peptide neurotransmitters to nerve endings seems to be different from that of classical neurotransmitter. This difference is shown schematically in Fig I. Peptides are probably produced only on the ribosome of the cell soma, possibly in the form of a larger precursor molecule without local synthesis in nerve endings. As no reuptake mechanisms of peptide in nerve ending, every single peptide molecule released from a nerve ending must be replaced by axonal transport. This comparatively inefficient and slow mechanism should be reflected in the dynamics of synaptic events at peptide synapses (Hökfelt et al. 1980). Perhaps peptides are released intermittently rather than tonically. Such an intermittent release may, however, be compensated by a

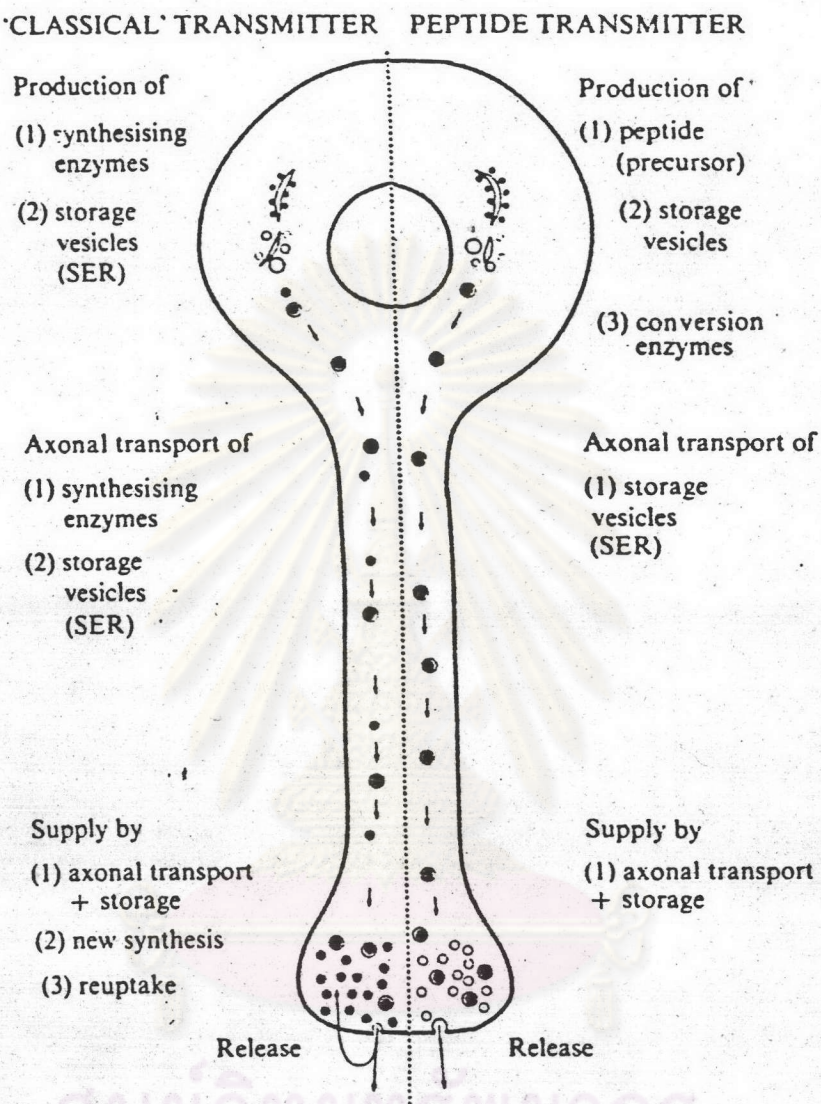

Figure 1 Schematic drawing of a neurone demonstrating some differences between a neurone using a "classical" Transmitter (left) and a peptide transmitter (right) (from Hökfelt et al, 1980).

long duration of action. The amounts of peptide released may also be much smaller than those of classical transmitters. This would be in line with the rather low concentrations of peptides found in the CNS. Furthermore, peptides may activate receptors at much low concentrations than the classical transmitters, a situation which may compensate for an inefficient replacement of released transmitters. It therefore seems that the neuropeptides may be chemical messengers of a character different from that of the classical transmitters. Many observations demonstrated that they play modulatory role rather than a transmitter role, by altering the neuronal response to the established neurotransmitters. (Barker, Smith and Neale, 1978 ; Haas, Felix, Celio and Inagami, 1980; Hökfelt et al. 1980 ; Iversen, 1982; Yarbrough, 1976). In most cases, however, the evidence is not yet strong enough to support the actual mechanism of neuropeptides.

The brain is therefore recognized as a target tissue of many peptide hormones. (Changaris, Severs, and Keil, 1978). When injected or infused into the central nervous system, one of these hormones, angiotensin II (A II), produces multiple biological effects including elevated blood pressure, drinking behavior, altered renal sodium excretion, and release of antidiuretic and adrenocorticotrophic hormones,

(Anderson, 1977; Fitzsimons, 1972; Maran and Yates, 1977; Severs and Daniels - Severs, 1973). The effect of AII on behavior is also interesting because it is one of several peptides which have been shown to have powerful behavioral actions (Phillips, 1978). The diversity of angiotensin's effects in the brain suggests that if this hormone were present, it would participate in the neurophysiology of multiple neuroanatomical compartments; The immunohistochemical results demonstrated angiotensin II (AII) in many cells and cell parts within many brain regions. (Changaris et al. 1978). Thus, the presence of immunoreactive products of AII has been demonstrated in hippocampus, striatum, cerebellum, combined hypothalamus : thalamus : septum : midbrain tissue, medulla and cortex. Hippocampus has the highest concentration and cortex has the lowest. (Sirett, Bray and Hubbard, 1981). In addition, synaptic boutons within the brainstem and cerebellum contain immunoractive AII (changaris et al. 1978). Both the deep cerebellar nuclei and cerebellar cortex showed AII immunoprecipitate. The deep cerebellar nuclei have the richest density of AII positive synapses within the fastigial and dentate nuclei, neurones are studded with AII positive synapses (Changaris et al. 1978). These neurones have the greatest density of immunoreactive AII, that visualized in the rat brain

(Changaris et al. 1978) . The granule cells are also densely positive for immunoreactive AII. Numerous AII positive fiber tracks can be visualized within the middle cerebellar peduncle. Scattered amidst the Purkinje cells (P cells) are large cell processes. These are 3 to 6 times larger than the synapses of cerebellar and tegmental nuclei. Occasionally these fibers course in apposition to Purkinje cells. (Changaris et al. 1978 ; Fig. 2 A,B). Most interestingly, angiotensinogen, the prohormone of angiotensin II, (Lewicki, Fallon and Printz, 1978), and converting enzyme (Yange and Neff, 1972), as well as AII recepter (Sirett, McLean, Bray, and Hubbard, 1977) are also found present within the central nervous system.

AII have several central actions, but recent studies have focused on the site at which this peptide acts on the brain. The possible postsynaptic actions of the peptide have now been studied extensively by electrophysiological techniques and in vivo recordings. AII was generally applied by use of microiontophoretic techniques. Direct iontophoretic application of AII on supraoptic neurosecretory cells as well as on neurones of subfornical organ produced an excitant effect. (Otsuka and Takahashi, 1977). However, all the actions are not specific for the subfornical organ and microiontophoretically applied AII also excites neurones

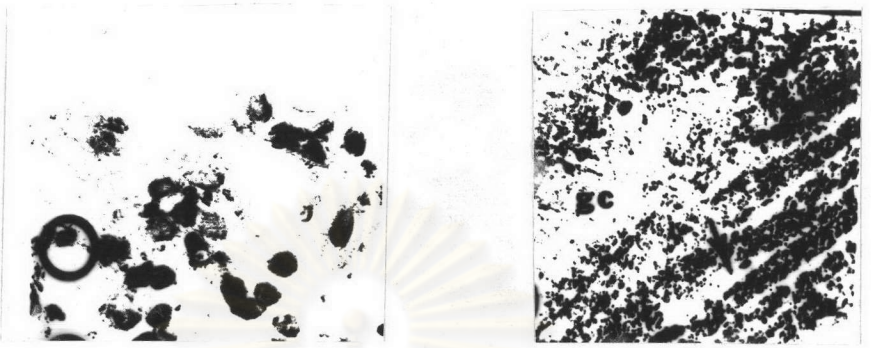

Figure 2 A Purkinje layer shows scattered AII positive cell processes (circle) which occasionally come in close apposition to purkinje neurons (hematoxylin; bright field). x 2,500.

Figure 2 B Cerebellar cortex and fibers to the middle cerebellar peduncle. AII positive fibers are seen within white matter of the middle cerebellar peduncle (arrow). The granule cells (gc) appear densely stained (dark field). x 600.
(From Changaris et al, 1978).

in the hypothalamus, thalamus, medial preoptic area, septum and cerebral cortex (Phillis and Limarcher, 1974).

In the cerebellum, a significant amount of AII (Sirett et at. 1981) as well as AII receptors (Sirett et al. 1977) are demonstrable by using radioimmunoassay and receptor binding techniques. In addition, immunocytochemical staining demonstrates the presence of AII containing fibers coursing in the cerebellar white matter and diverging within the granular layer to terminate as a dense collection of fibers surrounding the Purkinje cell somata (Changaris and Keil, 1978; Fig. 3A and B). This neurochemical findings suggest the neurotransmitter roles of AII in the cerebellar cortex with Purkinje cells being possible target neurones.

The action of AII on Purkinje cells as well as on other unidentified cerebellar cortical neurones have been demonstrated by means of extracellular recording with microiontophoretic techniques (Tongroach, Sanguanrungsirikul, Tantisira, and Kunluan, 1984). It were observed that AII consistently depressed spontaneous firing of Purkinje cell, whereas other unidentified neurones were unaffected. When tested against response of Purkinje cell to depressant putative

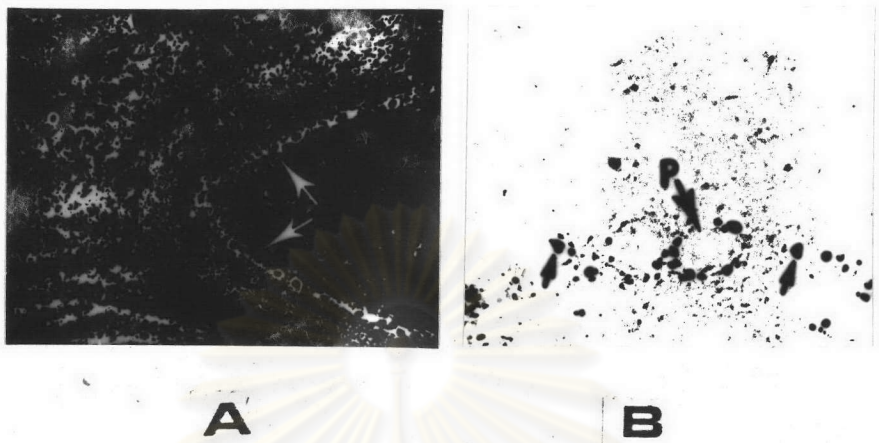

Figure 3. A and B A. Lowpower darkefield photomicrograph of the cerebellun stained for angiotensin II (A II) by the immunoperoxidase technique shows arching white fibers contiguous to the granular layer. Numerous white fibers are interspersed amidst Purkinje cell (arrows): magnification x 300.

B. Nomarsky interference photomicrograph of the cerebellar Purkinje layer shows perineuronal fibers rich with dark, immunoprecipitate, small arrows. The secion has no counterstain; Purkinje cells (P); magnification x 400 (From Changaris et al, 1978).

neurotransmitters, namely, GABA, glycine, taurine, 5-hydroxytryptamine and noradrenaline, it was observed that AII specifically enhanced depressant action of GABA, while the responses to other substances were unaffected. Both AII - induced depression of cell firing and the AII - induced enhancement of GABA depression were antagonized by a specific GABA antagonist, bicuculline methochloride; The results suggest that AII exerts an inhibitory action on Purkinje cells through its modulatory action on bicuculline - sensitive GABA receptors.

Neuropharmacology of Cerebellar Circuitry

The basic neuronal connections of the cerebellar cortex are summerized in Fig.4 and 5. In brief, there are two main sources of input to the cerebellar cortex: climbing and mossy fibers. Climbing fiber inputs exert a strong excitatory effect on single Purkinje cell. Whereas another source of excitation on this cell comes from mossy fiber input mediated through granule cells which originate the parallel fibers whose endings form synapses on Purkinje cell dendrites. The basket and stellate cells are also excited by granule cells via the parallel fibers, and their outputs inhibit Purkinje cell discharge. Golgi cells are excited by the mossy fiber collaterals and parallel fibers, and inhibited by

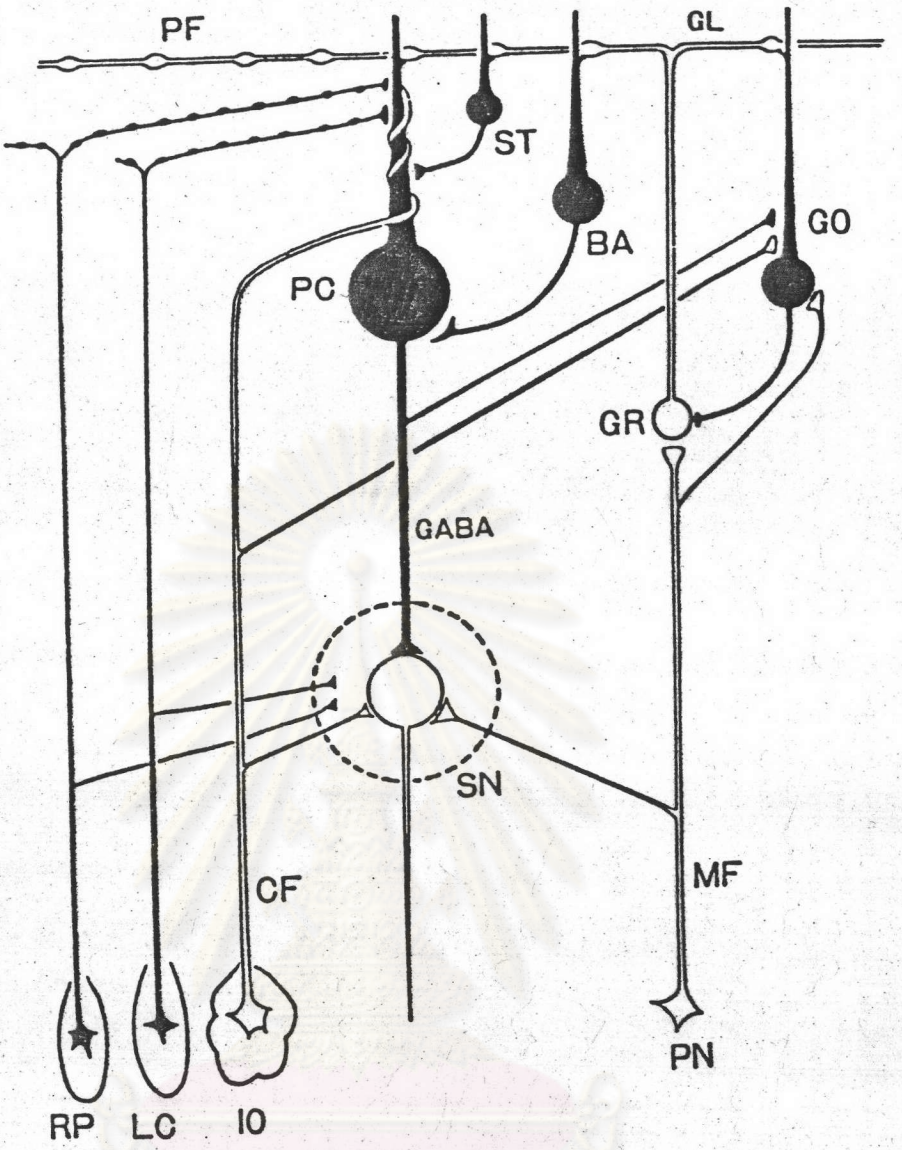

Figure 4 Basic neuronal circuitry and putative neurotransmitters in the cerebellum. PC, Purkinje cell; GO, Golgi cell; BA, basket cell; ST, stellate cell; GR, granule cell; PF, parallel fiber; MF, mossy fiber; CF, climbing fiber; SN, vestibular or cerebellar nucleur cell; PN, precerebellar neuron which issues mossy fiber; IO, Inferior olive; LC, locus coeruleus; RP, raphe nuclei. Inhibitory neurons and synapse are in black, and excitatory ones have been left unfilled, (From Ito, 1984).

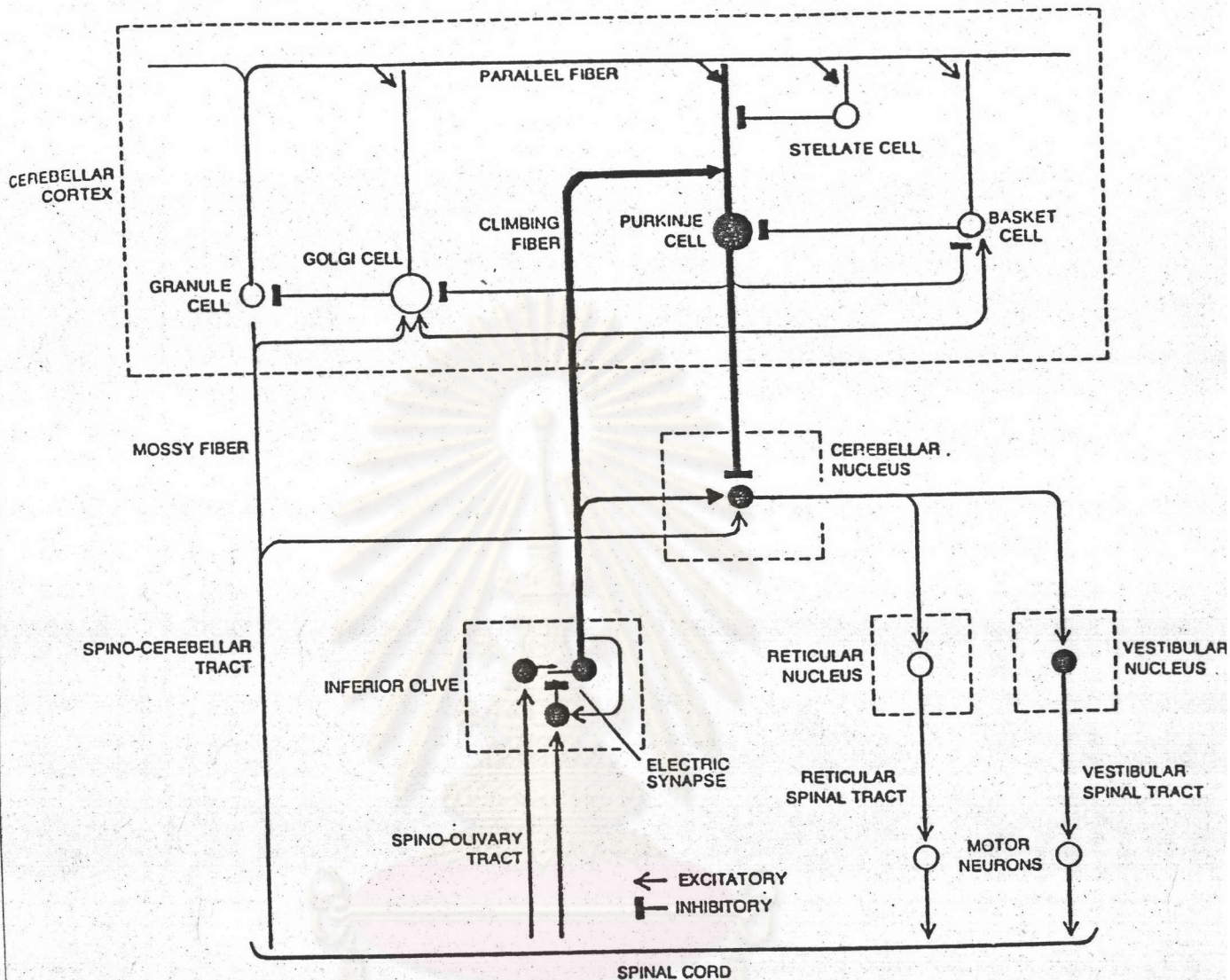

Figure 5 Wiring Diagram of the cerebellar cortex and the brain centers with which it communicates relates the structure of the nerve-cell circuits to their function ,(From Llinas, 1975).

Purkinje cell collaterals. These cells inhibit granule cells (for review see Eccles, 1973; Mouncastle, 1980; Szentagothai and Arbib, 1974).

A major inhibitory neurotransmitter in the cerebellum is gamma - aminobutyric acid (GABA). Evidence from several independent lines of research has strongly suggested that GABA is the transmitter substance for Purkinje cells in the cerebellum. These are inhibitory cells (Eccles, Ito, and Szentagothai, 1967), and GABA applied iontophoretically mimics their inhibitory action (Obata, Ito, Ochi, and Sato, 1967). This inhibition is blocked by bicuculline. (Kawaguchi, and Ono,1973). There is convincing evidence that GABA is present in Purkinje cell and released by this cell upon stimulation (Obata, et al. 1967; Otsuka, Obata, Miyata and Tanaka, 1971; Ribak, Vaughn, and Saito, 1978). Several evidences suggest that four of the five cell types in the cerebellar cortex may use GABA as their neurotransmitter, i.e., Purkinje cells, stellate cells, basket, cells and golgi cells (McGeer, Hattori, and McGeer, 1975). Stellate cells are thought to send inhibitory synapses to Purkinje cell dendrites (Anderson, Eccles, and Vourhoeve, 1964; Rushmer and Woodward, 1971; Woodword, Hoffer, Siggins, and Oliver, 1971). Basket cells send their inhibitory processes to

Purkinje cell bodies in a plane at right angles to the parallel fibers, while Golgi cells are typically located just below the Purkinje cell layer and their densely arborized axons inhibit granule cells. (Eccles and Szentagothai, 1967). Glutamic acid decarboxylase has been localized to nerve endings surrounding Purkinje cells in the region where basket nerve endings would be expected, although there is also a light diffuse reaction for glutamic acid decarboxylase over the Purkinje cell bodies (Saito, Matsuda, Roberts and Voughn, 1974). However, GABA may not be the only transmitter released by stellate cells. Selective destruction of these cells by X - irradiation is followed by a substantial reduction of taurine in the molecular layer (Nadi, MeBride, and Aprison, 1977). This would be consistent with the possibility that taurine is a transmitter released by at least some stellate cells, especially as Purkinji cell are quite sensitive to taurine (Okamoto, Quastel and Quastel, 1976), particularly when this is applied to their dendrites (Fredericson, Neuss, Morzorati ,and McBride, 1978).

The present study

According to electrophysiological study (Tongroach et al, 1984), iontophoretic application of

AII to Purkinje cells markedly enhanced the inhibitory action of GABA , while the responses to other substances were uneffected. The findings give rise to the possibility that synaptic action of this peptide, if any, may be modulatory on GABA actions. This possibility is further supported by the results that both depressant action of AII and AII - induced enhancement of GABA action were antagonized by a specific GABA antagonist, bicuculline methochloride (BMC) indicating that inhibition seen with AII application may be mediated through bicuculline - sensitive GABA receptors.

The present study is aimed at demonstrating in further details the action of AII on P cell as well as its interaction with GABA and its antagonists. Further attempt has also been made in order to elucidate possible modulatory action of the peptide. Two methods have been employed in this study. First, micro-iontophoresis was used in electrophysiological experiments in order to demonstrate interaction of the peptide with GABA. Second, measurement of GABA release by collection and biochemical methods, using high performance liquid chromatography (HPLC), have been performed to elucidate the effects of AII on the release amounts.