Chapter I

Introduction

It is generally agreed that information processing in the brain largely involves communication among neurones through the release of neurotransmitters at synapses. Until the 1960's the amines, acetylcholine (Ach), noradrenaline (NA) and 5-hydroxytryptamine (5-HT) were only well recognized transmitters. Then came an appreciation that amino acids such as γ-aminobutyric acid (GABA), glutamic acid (GLU), aspartic acid (ASP) and glycine (GLY) might serve as transmitters (McLennan, 1963). Although these seven established neurotransmitters, so-mentioned as "classical" neurotransmitters, seemed more than sufficients for coping with signalling interneuronal communications, further studies during the recent years reveal that the number of chemical messenger systems in the brain has expanded dramatically with the discovery of a new family of brain chemicals: the neuropeptides (Hökfelt, Johansson, Ljungdahl, Lundberg and Schultzberg, 1980; Iversen, 1979; Snyder, 1980).

If one were to establish neurotransmitter identity of a substance, certain criteria have to be considered. To recapitulate, briefly, it must be shown that the substance is present within the nerve cell and specifically in its presynaptic terminals, that the nerve cell can produce or accumulate the substance, and that the substance can be released by the nerve cell. The substance must be shown to mimic in every aspect the functional activity following

stimulation of the nerve that release it, including the magnitude and quality of changes in postsynaptic membrane conductance, and drugs which can stimulate or antagonize the effects of nerve fiber activation must have an identical influence on the effects of the substance applied exogenously to the target cell (for reveiw see Cooper, Bloom and Roth, 1982; Iversen, 1979).

In case of peptides, combined radioimmunoassay (the quantitative measurement) and immunocytochemistry (the qualitative distribution) have demonstrated their existence within the neurones. Some, such as the opioid peptide enkephalins, neurotensin, and substance P, were first isolated from the brain, whereas the others such as cholecystokinin (CCK) and vasoactive intestinal polypeptide (VIP) were known as intestinal hormones and later recognized as brain constituents (Hökfelt et al, 1980; Snyder 1980). Furthermore, the occurence of biologically active peptide with the same neurones containing classical neurotransmitter substances have also been observed (Schultberb and Hökfelt, 1982). Whereas for some peptides, there is a great deal of data to support the view that they behave as neurotransmitters, for others there is virtually none (Iversen, 1982; Kelly, 1982). It therefore seems that the neuropeptides may be chemical messengers of a character different from that of previously identified 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 Neal, 1978; Haas, Felix, Celio, and Inagami, 1980; Hökfelt et al 1980; Iversen, 1982; Phillis and Limacher, 1974; Yarbrough, 1976). In most cases, however, the evidence is not yet strong enough to support the actual mechanism of neuropeptides.

Of variety of the polypeptides, angiotensin II (A II), found firstly in the periphery as hormones, have also been demonstrated as neuronal constituent. Most of the peripheral actions of A II center around the regulation of the circulatory system, A II is a potent vasoconstrictor. It also causes renal sodium retention by stimulating aldosterone secretion from the adrenal cortex (for review see Mouncastle, 1980). More interestingly, an endogeneous brain renin-agiotensin system have been discovered (Phillips, 1978). In the rat brain, evidence for the existence of brain renin angiotensin system have been accumulating during the past recent years (Changaris, Severs and Keil, 1978; Inagami, Okamura, Hirose, Clemens and Yoko 1982). Thus, the presence of immunoreactive products of A II has been demonstrated in hippocampus, straiatum, cerebellum, combined hypothalamus: thalamus: septum: midbrain tissue, circumventricular organ, medulla and cortex (Sirett, Bray and Hubbarb, 1981). In addition, angiotensinogen (Lewicki, Fallon and Printz, 1978; Sernia and Reid, 1980; Wallis and Printz, 1980) and converting enzyme (Ganten, Minnich, Granger, Hayduk, Brecht, Barbeau, Boucher and Genest, 1971; Singh and McGeer, 1979) as well as A II receptors (Sirett, McLean, Bray and Hubbard, 1977) are also found present within the central nervous system.

A II have several centrals actions in addition to its peripheral vasoconstriction effect when injected or infused into the brain. These include stimulation of drinking, blood pressure increase and vasopressin release (Severs and Daniels-Severs,1973), and it may also cause adrenocorticotrophine releasing hormone (ACTH) release (Reid and Day, 1977). Recent studies have focused on the

site at which this peptide acts on the brain, it has been suggested that periventricular sites are involed in this response. Receptive sites for the drinking response have been proposed for the subfornical organ, preoptic region and tissue surrounding the anterior third ventricle. The pressure response has been reported to involve the area postrema, the subnucleus medialis in the cat and the anterior thrid ventricle region in the rat (Gronan and York, 1978).

In studying the effect of A II on single neurone, the possible post synaptic actions of the peptide have now been studied extensively by electrophysiological techniques and in vivo recordings, A II was generally applied by use of microiontophoretic techniques. Direct iontophoretic application of A II to supraoptic neurosecretory cells increase their discharge rate (Nicoll and Barker, 1971). Microiontophoretic studies (Felix and Akert, 1974) show the neurones of the subfornical organ to be excited by A II. The effect is dose dependent and appear to be specific since the neurones do not respond to bradykinin, eledosin or physalaemin, while the same neurones were blocked by completitive A II antagonist saralasin (See below; Phillips and Felix, 1976). However, the actions of A II are not specific for the subfornical organ and microiontophoretically applied A II also excites in the hypothalamus, thalamus, medial preoptic area, septum and cerebral cortex (Phillis and Limarcher, 1974).

In hippocampus slice preparation, Hass, Felix, Celio and Inagami (1980), have shown that bath application of A II (1-10 μmole/l) caused a dose dependent increase in amplitude of the extracellularly recorded excitatory field potential. This effect was specificly blocked by saralasin [(Sar', Thr^8) A II]. A II had no effect on antidromic

field potential but during double shock studies blocked the reduction of the second response cause by recurrent inhibition. Intracellular experiments on 9 neurones confirmed that the excitatory action of A II was accompanied by a depolarization and prolongation of the synaptically evoked excitatory postsynaptic potential (EPSP) and reduction in the duration of the accompanying inhibitory postsynaptic potential (IPSP). Since these effect were all blocked by saralasin and accompanied by little or no change in membrane resistance, Haas et al (1980) argue that the apparent excitation evoked by A II could be a results of disinhibition. Haas et al (1980) go on to suggest that the degree of excitation evoked by one transmitter might be modulated by a presynaptic action of the peptide on adjadent nerve terminals. However, this suggestion needed to be confirmed. In consideration of histochemical and electrophysiological studies, these findings prompt the suggestion that A II may have transmitter functions in the central nervous system.

In the cerebellum, a significant amount of A II (Sirett, Bray and Hubbard, 1981) as well as A II receptors (Sirett, McLean, Bray and Hubbard, 1977) are demonstrable using radioimmunoassay and receptor binding techniques. Immunocytochemical staining demonstrates the presence of A II containing fiber 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, Severs and Keil, 1978; Figure 1 A and B). This neurochemical findings suggest the transmitter roles of A II in the cerebellar cortex with Purkinje cells being possible target neurones.

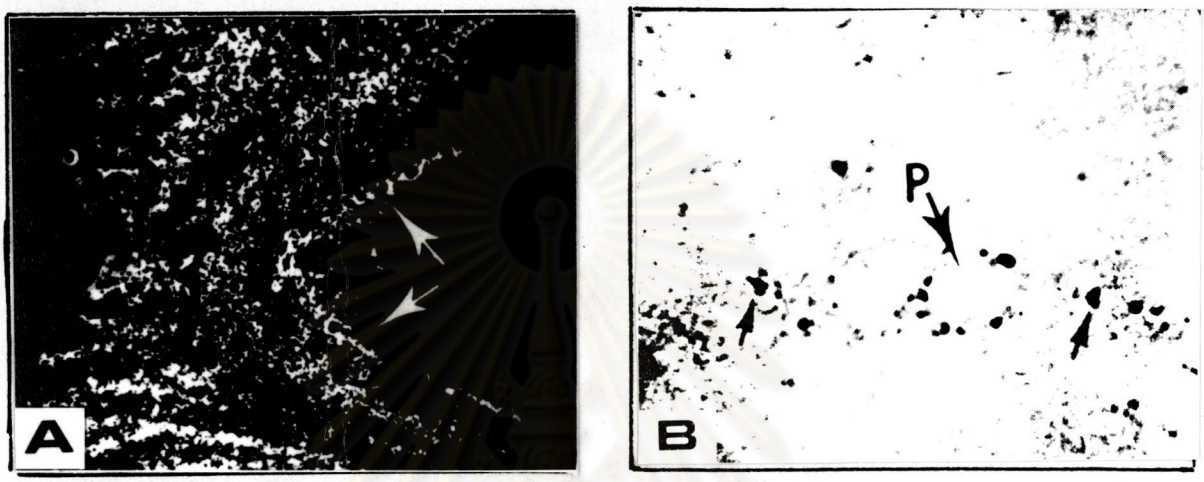

Figure 1. A and B

A. Low power darkfield photomicrograph of the cerebellum 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 100.

B. Nemarsky interference photomicrograph of the cerebellar Purkinje layer shows perineuronal fibers rich with dark immunoprecipitate, small arrows. The section has no counterstain; Purkinje cells (P); magnification x 400.

(From Changaris et al, 1978).

The basic neuronal connections of the cerebellar cortex are summerized in Figure 2 and 3. In brief, there are two main sources of input to the cerebellar cortex: climbing fibers 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 fibers 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 Purkinje cell collaterals. These cells inhibit granule cells (for reviews see Eccles, 1973; Mouncastle, 1980; Szentagothai and Arbib, 1974).

γ-Aminobutyric acid (GABA) is known to be a major inhibitory neurotransmitter in the cerebellum. There is convincing evidence that GABA is present in Purkinje cells and released by this cell upon stimulation (Obata, Ito, Ochi and Sato, 1967; Otsuka, Obata, Miyata and Tanaka, 1971; Ribak, Vaughn and Sato, 1978). In addition, immunocytochemical (McLaughlin, Wood, Saito, Barber, Vaughn and Roberts, 1974) and neuropharmacological (Bisti, Iosif and Strata, 1971) evidence indicates that GABA may also be the inhibitory transmitter released from golgi cells, basket cells, and possibly from some stallet cells. 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, McBride and Aprison, 1977). This would be consistent with the possibility that taurine is a (the) transmitter

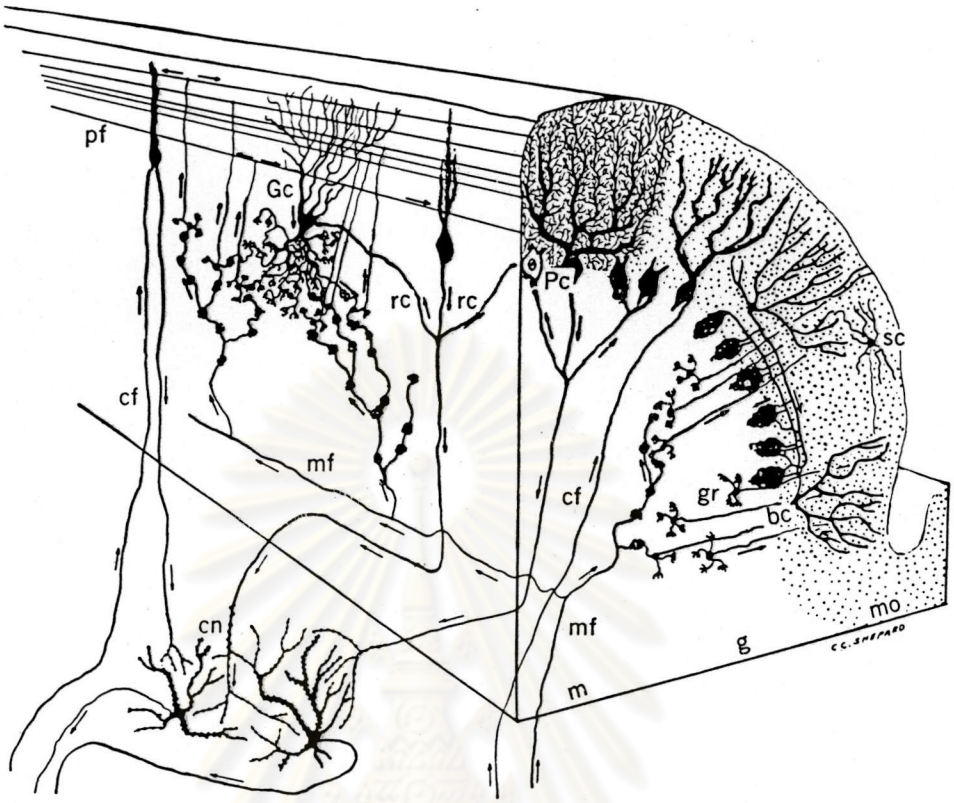

Figure 2. Schematic view of cerebellar folium. bc, Basket cells; cf, climbing fiber; cn, deep cerebellar nuclei; g, granular layer; Gc, Golgi cell; gr, granule cell; m, medullary layer (white matter); mf, mossy fiber; mo, molecular layer; Pc, Purkinje cell; pf, parallel fiber; rc, recurrent collateral; sc, stellate cell. (From Mouncastle, 1980).

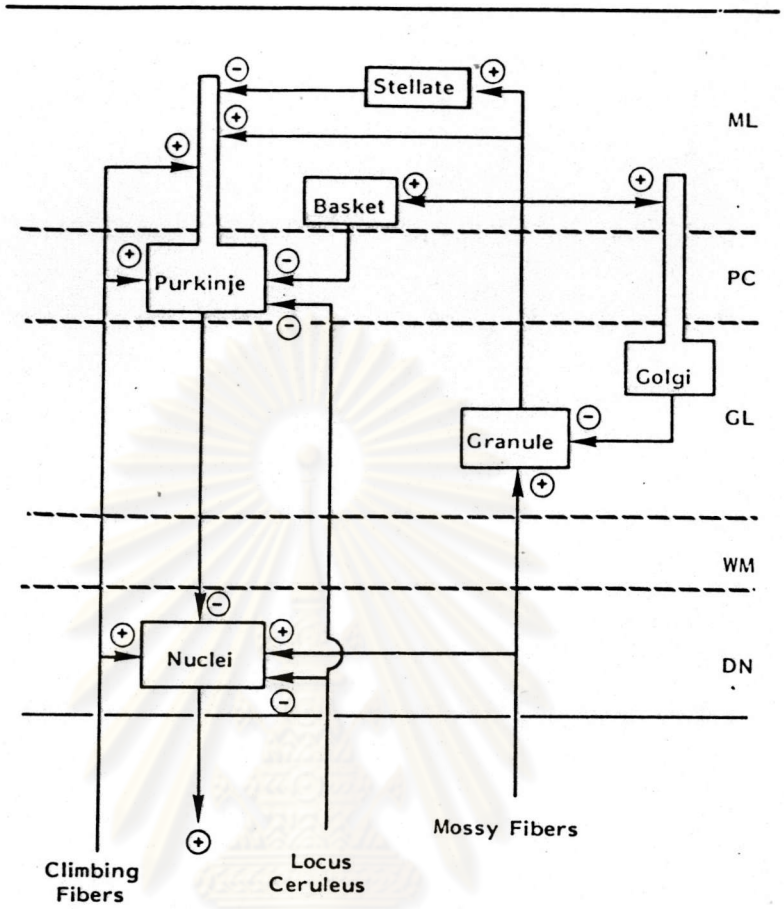

Figure 3. Simplified block diagram of cerebellar circuits. Plus signs refer to excitation and minus signs to inhibition. The molecular layer (ML), Purkinje cell layer (PC), granular layer (GL), white matter (WM), and deep nuclei (DN) are indicated. (From McBride and Frederickson, 1980).

released by at least some stellate cells, especially as Purkinje cell are quite sensitive to taurine (Okamoto, Quastel and Quastel, 1976) particularly when this is applied to their dendrites (Frederickson, Neuss, Morzorati and McBride, 1978).

Several kinds of evidence point to glutamate as the excitatory transmitter released by the granule cell parallel fibers terminated on Purkinje cell dendrites. Purkinje cell are highly sensitive to L-glutamate particularly in this regions of dendrites (Chujo, Yamada and Yamamoto, 1975). In addition, there is high concentration of glutamate in the cerebellum, especially in the molecular and the granular layers (Nadi et al, 1977). Selective reduction in tissue (or synaptosomal) glutamate contents is observed in animals that lack granule cells and their axon owing to genetic defect (Roffer-Tarlov, Beart, Gorman and Sidman, 1979; Roffer-Tarlov and Sidman, 1978), or as a consequence of a viral infection (Young, Oster-Granite, Herndon and Snyder 1974), or after selective x-irradiation (Rohde, Rea, Simon and McBride, 1979).

There is now extremely good evidence that noradrenaline (NA) is an inhibitory transmitter on cerebellar Purkinje cell and 5-hydroxytryptamine (5-HT) may also be a transmitter in the cerebellar cortex. Histochemical and autoradiographic studied of the rat cerebellum have revealed the presence of NA and 5-HT containing nerve fibers in molecular and Purkinje cell layers both in vitro and in vivo (Tebecis, 1974). Noradrenergic pathway originates predominantly from the locus coeruleus (Olson and Fuxe, 1971), and 5-hydroxytryptaminergic pathway presumably from the raphe' nuclei (Dalhlstrom and Fuxes, 1965). Direct iontophoretic applications of

NA and 5-HT to Purkinje cells decrease their discharge rate (Kawamura and Provini, 1970), and single or repetitive stimulation of locus coeruleus inhibit the firing of most Purkinje cells (Bloom, Hoffer and Siggins, 1972).

Since evidence from neurochemical study shows that glycine (GLY) can be taken up by golgi cells (Krnjevic, 1982), possibility exist that this amino acid may be another transmitter in this brain area. Iontophoretic application of GLY depressed the firing of Purkinje cells but the response was less effective than that of GABA (Kawamura and Provini, 1970). In consideration of aspartic acid (ASP), Purkinje cells can be strongly excited by this amino acid, although neurochemical evidence make it unlikely that it is released by parallel fiber (Krnjevic, 1982). However, when climbing fibers are destroyed selectively either by disease in human (Perry, Currier, Hansen and MacLean, 1977), or by the action of 3-acetyl-pyridine in rats (Nadi, Kanter, McBride and Aprison, 1977; Rea, McBride and Rohde, 1980), the most significant observation is a consistent reduction in ASP. Hence ASP can be considerated the most possible contender for being climbing fiber's transmitter.

The present study is undertaken to investigate the actions of A II on Purkinje cells as well as on other unidentified cerebellar cortical neurones by means of extracellular recording in conjunction with microiontophoretic techniques. In veiw of current ideas obout possible modulatory function of polypeptide neurotransmitters (e.g. Hökfelt et al, 1980), the present experiments are also designed to test the effects of A II on neuronal responses induced by "classical" neurotransmitter substances reportedly reactive in the cerebellum (see above).