Chapter 4

Discussion

Several studies (Otsuka et al. 1977; Phillis et at. 1974; Tongroach et al. 1984) have shown that the use of electrophysiological techniques to study actions of AII on single neurones are of distinct advantage. The techniques measure directly the electrical activity of a single neurone which is an important functional parameter of the nervous system. Other method to look for the action of AII on endogeneous amino acids employed neurochemical methods which involve collection of endogeneous amino acids functioning as neurotransmitters in a specific brain tissue, the cerebellar cortex, using push-pull perfusion technique.

Results in the present study are complementary to biochemical (Sirett et al. 1981) and anatomical (Changaris et al. 1978) observations which demonstrate the presence of AII in the cerebellum. Immumocytochemical studies show AII containing fibers crossing the white matter and the granular layer of the cerebellum to terminate on the Purkinje cell layer as a dense collection of fibers surrounding the Purkinje cell (Changaris et al. 1978; Sirett et al.1977). AII binding was found not only in the hypothalamus, and midbrain

but also in the cerebellum. It is tempting to suggest from the forthmentioned studies that AII may have neurotransmitter functions in the cerebellum with Purkinje cells being the possible target neurones.

In the cerebellum, the cellular actions of this peptide has to be established (Tongroach et al. 1984). Thus, it has been demonstrated that Purkinje cells, but not other unidentified cortical neurones, consistently respond to iontophoretic application of AII by a reduced firing rate. Such response confirms the presence of AII recepter, and suggest further that these receptors may be localized on the Purkinje cell membrane, or at least, on neuronal elements functionally connected to the Purkinje cells. The results are also consistent with immunohistochemical data (Changaris et al. 1978). In addition to its inhibitory effects on Purkinje cells, AII specifically enhances inhibitory action of GABA, gives rise to the possibility that synaptic action of this peptide, if any, may be modulatory on GABA actions. This possibility was supported by the results that both depressant actions of AII and AII - induced enhancement of GABA action were antagonized by a specific GABA antagonist,BMC, indicating that inhibition seen with AII application may be mediated through BMC - sensitive GABA receptors (Tongroach et al. 1984).

In this study, the inhibitory actions of AII was anta-gonized by saralasin, AII antagonist , while the inbition of neuronal activity caused by GABA was uneffected. In addition , inhibitory action of AII and GABA was potentiated by AOAA applied microiontophoretically. It was suggested that AOAA could promote colletion of released transmitter by causing either less effective inactivation of GABA or excessive production and release of the amino acid . Both of these possibilities must exist since inhibition of the enzyme is associated with a rise in the brain GABA level. By analogy with the neuromuscular junction, impairment of GABA inactivation would be expected to enhance the intensity and duration of synaptically evoked inhibition (Gottesfeld et al. 1972).

From the study, it seems that AOAA may elevated the concentration of endogeneous GABA, so that it potentiated inhibitory action of GABA applied microiontophoretically. Other evidence showed that following systemic administration of AOAA, the GABA level was increase and enhancement of inhibitory action of GABA occured within 1 hr. This suggests that AOAA may specifically increase the sensitivity of GABA receptors independently of any effect on GABA - T. AOAA could also cause cells to become more susceptible to

inhibition by some indirect non-specific action on the cortical blood supply or the level of some essential metabolite (Gottesfeld et al. 1972). However, in this study, AOAA enhanced inhibitory action of GABA and A II, preliminary study showed that both A II induced inhibition and A II induced enhancement of GABA's action were antagonized by BMC, gives rise to the suggestion that the inhibitory actions of AII may mediate through GABA ergic mechanism, by enhancing an increase in endogeneous GABA release.

Urethane was chosen as anesthetic electrophysiological study because it does not modify either uptake or release of GABA from rat thalamic or cortical slices (Maggi C.A. and A. meli, 1986). In biochemical study, however, preliminary study showed that amino acid release in urethane anesthetized animal was very small in amount so that detection became troublesome. Nembutal was chosen in preference to urethane because many literatures showed that it enhance GABA release (Van Gilder J.C. and O'Leary J.L., 1970; Bowery and Dray, 1977; Evans R.H., 1979; Study R.E. and Barker J.L., 1981)

Measurement of the in vivo release of endogeneous amino acids has several advantage over technique for measuring in vitro release of endogeneous

amino acid previously added to the tissue slices or homogenates. Firstly, in-vitro experiments require dissection of the brain tissue, thus disrupting all neuronal connections. Secondaly , in vitro experiment requires preloading of the tissue with labelled amino acids and measurement of the subsequent release. This technique may result in release of endogeneous amino acids from sites other than nerve terminals.

The present study employs neurochemical methods which involve collection of endogeneous amino acids using push-pull perfusion technique. Several investigators (Yask and Yamamura, 1973,1974 ; McLennan,1964) have employed this method successfully in studying the release of putative neurotransmitters. The HPLC method employed in this experiment is well suited for determination of the amino acids in perfusate sample because it is easy to perform and used, and is thus an excellent tool for gerneral studies of amino acids metabolism. The O - phthaldehyde (OPA) fluorometric derivatization provides high sensitivity and the described method gives reproducible results with minimal sample preparation. Furthermore, determination of standard curves using known amounts of amino acids suggests that the results obtained from our method is linear over a wide range (10 pmol - 1600

pmol/ml.). This linearity has been reported in the previous studies even over a wider range (10 -5000 pmol/ml.) (Van et al,. 1978, 1979; Ischida, Fujita and Asai, 1981) .

In the present experiment, amino acids detected in the push-pull perfusate are aspartate (Asp.), glutamate (glu), serine (ser), glutamine (glu - NH), glycine (gly), taurine (tau), alanine (ala). and GABA. Of the substance listed, two (asp and glu) are classified as putative excitatory neurotransmitters and three (tau, gly, and GABA) are putative inhibitory neurotransmitters (Fagg and Foster, 1983).

A general increase in efflux of amino acids, both in vivo and in vitro, have been reported to occure after potssium depolarization. In this experiment, a high concentration of K^+ (100 mM) in the artificial CSF has been shown to increase the efflux of endogeneous amino acids in the perfusate. With the presence of AII in artificial CSF, the high-K^+ induced efflux of endogeneous amino acids is markedly potentiated .

Conclusion

With reference to GABA, inhibitory action on Purkinje cells was potentiated by AII applied

microiontophoretically. These actions were antagonized by a specific GABA antagonist, bicuculline methochloride. This may suggest that AII exerts an inhibitory action on Purkinje cells through its modulatory action on bicuculline - sensitive GABA receptors. In addition to modulation on GABA receptors, the inhibitory action of AII and GABA were potentiated by AOAA , and the actions were antogonized by saralasin, the antogonist of AII, without any effects on action of GABA. It may be reasonable to suggest that AII produces inhibitory effects by inducing an increase in endogeneous GABA release from the nerve terminals. Such possibility has been explored in the release experiment and is confirmed by the finding that GABA release is indeed enhanced. The present study shows a good model for the mode of action of a peptide in the cerebellar cortex in the view of the current idea about possible modulatory function of peptide neurotransmitter.