Chapter IV

Disscussion and Conclusion

The use of electrophysiological techniques to study actions of A II on single neurones are of distinct advantage. The techniques measure directly the electrical activity of a single neurone which is important functional parameter of the nervous system. A convenient method to look for A II sensitive neurones seems to be to follow single neurone discharges, under the influence of microiontophoretically ejected A II from a multibarrel microelectrode.

Results in the present study are complementary to biochemical (Sirett et al 1981) and anatomical (Changaris et al 1978) observations which demonstrates the presence of A II in the cerebellum. Immunocyto-chemical studies show A II 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) indentified high affinity binding sites for ^{125}I-A II on rat brain membranes. A II binding was found not to be only in the thalamus, hypothalamus and midbrain, but also in the cerebellum. It is tempting to suggest from the forthmentioned studies that A II may have neurotransmitter functions in the cerebellum with Purkinje cells being the possible target neurones.

If we were to suggest that A II is present in the cerebellum as a neurotransmitter, then the cellular actions of this peptide has to be established. Such data have been accumulated in the present study. Iontophoretic applications of A II consistently produced depression of the spontaneous discharge in a dose-dependent manner (Figure 6 A), this suggests that the mode of synaptic function of this peptide on Purkinje cell may be inhibitory in nature. It is noteworthy that this finding is at variance with the reported excitatory action of A II in the other brain areas. For example, microiontophoretic studies show the neurone of the subfornical organ to be excited by A II and the effect is dose-dependent (Felix and Akert, 1974). Direct iontophoretic applications of A II to supraoptic neurosecretory cells increase their discharge rate (Nicoll and Barker, 1971). The peptide also excites neurones in the hypothalamus, thalamus, medial preoptic area, septum and cerebral cortex (Phillis and Limarcher, 1974). The discrepancies of the results demonstrated in this study therefore suggest a different mode of action of this peptide in the cerebellum, as oppose to the documented excitation actions in the other brain areas.

The findings in these present study that only Purkinje cells but not unidentified nonPurkinje neurones responded to iontophoretic application of A II (Figure 6 A and B) and the depression action of A II on Purkinje cells were antagonized by the specific A II antagonist saralasin (Figure 7) confirms the presence of A II receptors, and suggest further that these receptors may be localized on Purkinje cell membrane or at least on neuronal elements functionally connecting with the Purkinje cell.

The major results of the study were that, at subthreshold doses, A II selectively potentiates GABA-induced depression of Purkinje cell firings without showing any appreciable effects on the actions of other depressant amino acid neurotransmitters (Figure 9), excitant amino acid neurotransmitters (Figure 12) and depressant amine neurotransmitters (Figure 10 and 11) may bear a significant implication on the role of the peptide in the cerebellum. Possibility arises that synaptic function of A II, if any, in the cerebellum may be modulatory to GABA action. The results also demonstrated that the enhancement of GABA-induced inhibition by A II was antagonized by competitive A II antagonist saralasin (Figure 13). This suggests the necessity of A II receptors for this action. Another crucial point may be indicated by the observation this enhancement was obolished by a GABA antagonist BMC (Figure 15), which indicates that the observed A II actions were mediated via a GABA mechanism rather than through an additive inhibitory effect of A II.

Importantly, as shown in Figure 14, BMC antagonized not only the action of GABA, but also that of A II. This observation supports the involement of GABA as a mediator of the A II actions. On the other hand, saralasin which antagonized A II action does not show any antagonism to GABA (Figure 8). Therefore the possibility that A II may depress the neurone by simply acting on the same receptor as GABA can be excluded.

Unfortunately, data accumulated by the discipline used in this study fail to elucidate the mechanism of the modulatory effects of A II. Nevertheles, one can hypothesise that there may be two possible sites of action of A II in this observation; first, a postsynaptic site

through which A II may alter the efficacy of GABA-receptor interaction, and second, a presynaptic site through which A II may act by modulating GABA release. The presence of presynaptic A II receptor and falicitory effect on transmitter release of A II have been demonstrated in noradrenergic nerve endings (Langer, 1977; Mustafa, Lokhandwala, Amelang and Buckley, 1978; Starke, 1970; Zimmerman, 1973). It remains to be demonstrated whether the same kind of receptor would exist in the cerebellum. Although it is not clear in the light of the present results how the actual mechanism of this GABA involvement may be, the present study shows a good model for the mode of action of a peptide in the brain in the view of the current idea about possible modulatory function of peptide neurotransmitter.