CHAPTER V

DISCUSSION

In the present study, microdialysis technique, has proved to be a useful *in vivo* technique when studying events in the extracellular space, and has been used to investigate the release of endogenous amino acids in the vestibular nuclei. This technique eliminates many of the problems associated with push-pull perfusion and the need to clean up the sample before the HPLC injection. Tissue damage because the flow and associated turbulence usually found in the case of push-pull perfusion is also minimized (Wages, Church and Justice, 1986; Benveniste and Huttemeier, 1990). This technique, however, has its limitations. For example, microdialysis does not permit measurement of actual transmitter concentrations and their changes in the synaptic cleft during neurotransmission but only an average of these over a certain period of time required for suitable sample collection (Johnson and Justice, 1983). However, several investigators have employed this method successfully in studying the release of dopamine, norepinephrine, serotonin, acetylcholine and certain amino acids, including this study.

The HPLC system with pre-column derivatization method described is an excellent tool and suitable for the analysis of endogenous amino acids in the perfusate in picomole range. The described system allows a fast, accurate and vary sensitive determination of amino acids. The advantage of OPA method is

due to the fact that it rapidly reacts specifically with primary amines to form products that can be detected by a sensitive fluorescence detector (Hill et al., 1979; Buck and Krummen, 1984; Sanberg et al., 1987). The precision of this system is acceptable and the standard curve for each amino acids are satisfactorily linear through out the concentration range examined (50-2500 pmol/50 µl).

In this study, the levels of endogenous amino acids, i.e Asp, Glu, Ser, Gln, Gly, Tau, Ala and GABA, in the vestibular nuclei were measured in both normal and lesioned rats. It is generally assumed that GABA and, to a lesser extent, Gly are the major inhibitory transmitter and that Glu and Asp considered to be major excitatory neurotransmitters in mammalian central nervous system (Hamberger et al., 1979; Fonnum, 1984; McGeer and Eccles, 1986).

A general increase in efflux of amino acids, both in vivo and vitro, have been reported to occur after potassium depolarization. In this experiment, a high concentration of K^+ (100 mM) in the artificial CSF has been shown to increase the efflux of endogenous amino acids in the perfusate, two (Asp and Glu) especially showed a remarkable K^+-evoked release. This result is consistent with previous study by Warunee (1987), who also observed endogenous amino acid release in vestibular nuclei with push-pull perfusion technique. A note of precaution, however, suggest from some studies which have shown that release by potassium depolarization is not entirely specific for

neurotransmitters and that release can occur from sources other than pre-synaptic terminals

Following electrical nerve stimulation, the release of Asp and Glu increased significantly while Ser and Tau also showed some increase but the change was not statistically significant. Other amino acid did not show any change in the release. This finding demonstrated that Glu and/or Asp probably play a neurotransmitter role in afferent vestibular. These results together with other electrophysiological and biochemical evidence support the role of Glu, or a glutamate like substance as the major excitatory synaptic transmitter of vestibular nerve (Dememes, Raymond and Sans, 1984; Raymond et al., 1984; Sangchantra, 1986; Cochran, Kasik and Precht, 1987; Warunee, 1987; Touati, Raymond and Dememes, 1989).

To be qualified as the transmitter of a given pathway, a substance must be satisfy several classical criteria. Changes in transmitter release following specific destruction of know or suspected brain pathways in which it is involved may be a useful supplementary criterion (McGeer and Eccles, 1986). In this study, it was found that unilateral vestibular nerve lesion causes a characteristic pattern of acute disorganization of posture and movement. This finding was similar to those observed in unilateral labyrinthectomy or vestibular neurectomy which produce postural asymmetries, characterized by a head and body tilt toward the side of the lesion, and extension of the limbs on the intact and a flexion on the operated side (Xerri et al., 1983;

Galiana, Flohr and Jones, 1984; Waele et al., 1989; Newland and Perachino, 1990; Waele et al., 1990). In addition, it also causes the reduction in the normal level of amino acids within the lesioned vestibular nuclei (Henley and Igarashi, 1991).

In acute lesioned rats, slight initial increase was observed in the case of Asp, Glu Gly and Tau, with subsequent decrease found gradually following prolong sample collection. Three days after lesion, there was marked reduction in Glu, Gln, Ala and Tau levels in vestibular nuclei of the lesion side compared to those of the contralateral side ($p<0.05$), while the others such as Asp, Ser, Gly and GABA also decreased but the changes were not statistically significant. Furthermore, it was found that in lesioned rats the decrease in Glu release after K^+-evoked release were greatest, indicating that Glu may be a more likely candidate for the vestibular nerve neurotransmitter, rather than Asp.

Although the difference in the release of Glu from vestibular nucleus of the lesioned side and the contralateral side was not different statistically at 7 days after unilateral nerve lesion, there was, however, a slight trend for mean Glu levels to be decreased, while mean Asp levels showed a slight increasing trend. These quantitative finding of Glu and Asp levels are consistent with reports from Henley and Igarashi (1990) which showed in homogenate of vestibular nuclei of Squirrel monkeys 10 months after unilateral labyrinthectomy. The recovery found after 7 days suggests that the lesion of vestibular nerve may be incomplete so lesioned nerve still has the capacity to transmit

excitation centripetally and contribute to the compensation. Jensen (1983) reported that after vestibular deafferentation, the vestibular nerve does not completely degenerate and the compensatory excitation exerted by the nerve's remaining spike activity appears to be small. However, it is possible that there are enhancement of other glutaminergic input which come through spinal ascending system (Dieringer, Kunzle and Precht, 1984) and vestibular commissural system (Dieringer and Precht, 1979; Galiana, Flohr and Jones, 1984; Fetter and Zee, 1988; Knopfel and Dieringer, 1988) to the vestibular nuclei during the acute phase of vestibular compensation; a time during which system imbalance is dominant. Besides, many investigations have shown that excitatory synaptic inputs to vestibular neurons are mediated mainly through non-NMDA type of EAA receptors from vestibular afferents and through NMDA as well as non-NMDA types of EAA receptors from commissures (Cochran, Kasik and Precht, 1987; Lewis, Gallagher and Shinnick-Gallagher, 1987; Lewis etal., 1989; Doi, Tsumoto and Matsunaga, 1990). Therefore, it is concluded that glutamate is the neurotransmitter in primary vestibular afferent while both glutamate and aspartate acts as the neurotransmitters in vestibular commissural system.