Chapter 4

Discussion

Recent evidence suggests that glutamate and aspartate might function as neurotransmitters in the central nervous system. One of the central requirement for transmitter identification is to demonstrate that stimulation of presynaptic axons evokes a synaptic release of the substance which the stimulus-secretion coupling process is dependent on the presence of extracellular calcium ions. Numerous studies have demonstrated the release of radiolabelled amino acids from in vitro tissue slice preparation upon stimulation (Katz, Chase and Kopin, 1969; Srinivasan, Neal and Mitchell, 1969 ; Hammerstad, Murray and Culter, 1971). However experiments in which it has been possible to detect such a release in vivo are few (Jasper and Koyama, 1969; Jasper, Khan and Elliott, 1965; Iversen, Mitchell and Srinivasan, 1971). In some studies, endogenous amino acids released in superfusate collected from in vivo preparation was estimated after electrical stimulation (Obata and Takeda, 1969; Iversen et al., 1971; Van der Heyden, Venema and Korf, 1979).

In this experiment, we determine the change of endogenous amino acids release in a specific brain area, the vestibular nuclei, by using a push-pull cannula perfusion. Recently, push-pull cannula have been

sucessfully used for measuring the in vivo release of endogenous amino acids such as GABA , glycine, 5-hydroxytryptamine under normal condition and neuronal stimulation in a deep brain structure and with minimal tissue damage. (Yask and Yamamura, 1974; Nieoullon , cheramy and Glowinski, 1977)

The HPLC method for the quantitative analysis of the endogenous amino acids in the perfusate described is an excellent tool because it is easy and efficient、to perform. Only small amounts (upto 200 µl) of samples are required for each estimation. For the fluorimetric detection we employed conditions similar to those described previously (Lenda and Svenneby, 1980; Roth and Hampai, 1983). Precolumn formation of amino acids derivatives, followed by HPLC separation reduces chromatographic analysis time and provides greater sensitive than the postcolumn method (Gardner and Miller., 1980). The standard curve for each amino acids are satisfactorily linear throughout the concentration range examined (40-1600 pmol/ml). This liniarity 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 perfusate are aspartate (asp), glutamate (glu), serine (ser), glutamine (glu-NH_2), glycine (gly), taurine (tau), alanine (ala), and γ-aminobutyric acid

(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 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. During calcium-free superfusion, the potassium stimulated release of endogenous amino acids was abolished. While the release of amino acids is not effected by lower K^+ concentration (50 mM) with presence of Ca^+. The present study shows that appropriate depolarizing stimuli specifically alter the release of amino acids. The release of amino acids is a Ca^+-dependent. This is a property shared by all transmitter release mechanisms so far studied (Simpson, 1968; Rubin, 1970; Fagg and Lane, 1979).

Many studies have shown that release by potassium depolarization is not entirely specific for neurotransmitter and that release can occures from sources other than presynaptic terminal (Minchin, 1974; 1975; Robert, 1974; Reubi and Cuenod, 1976). In addition,ipsilateral vestibular nerve was stimulated

with direct electrical stimulation. In all experiment in which stimulating electrode and push-pull cannula were precisely located the significant increase in aspartate and glutamate, excitatory neurotransmitter, was observed in perfusate while those of another amino acids did not change. It was observed that serine and alanine also increased but the change was not statistically significant. Our results are in good correlation with electrophysiological finding on the characteristic of the vestibular unit firing pattern elicited by vestibular nerve stimulation.

The present study suggests that the afferent vestibular nerve may use aspartate and glutamate as neurotransmitters as it has been recently hypothesized from data based on the retrograde transport of [^{3}H]-D-Asp and [^{3}H]-Glu (Dememes et al., 1984; Raymond et al., 1984).It is interesting to notice that some study has suggested that glutamate probably act as the putative neurotransmitter in the afferent vestibular pathways (Saengchantra, 1986).

Further investigation may be performed which showed involve determination of the changes in amino acids released following vestibular nerve lesion.