Chapter 4

Discussion

Several studies have shown that when 3-acetylpyridine (3-AP) is injected intraperitoneally into rats symptoms characterizing severe impairment of cerebellar functions were observed. Recent histological studies of several areas of the CNS of rats treated with 3-AP show partial degeneration of facial, hypoglossal and ambiguus nuclei and complete degeneration of the inferior olive. Degeneration of the inferior olive leads to destruction of the climbing fibers projection from this structure into the cerebellum. In our study, akinesia characterized by "mud-walking" movement was observed 3 days after the injection. This symptomatic change was taken as an indication to suggest olivary destruction, as described by Desclin and Escubi (1974). The specific destruction of the climbing fibers, which constitutes a major excitatory input to the cerebellum may provide a tool for the identification of the neurotransmitter released from the olivo-cerebellar projection.

The present study employs neurochemical methods which involve collection of endogeneous amino acids functioning as neurotransmitters in a specific brain tissue, the vestibular nuclei, using push-pull perfusion technique. Several investigators have employed this method successfully in studying the release of putative neurotransmitters such as acetylcholine, norepinephrine and 5-hydroxytryptamine. The use of the push-pull canula in physiological experiment requires that reliable and reproducible data be obtaind which reflect the changing levels of materials in deep brain structures (Yask and Yamamura,1974).

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,no sample preparation and is thus an excellent tool for general studies of amino acids metabolism. The O-phthaldehyde (OPA) fluorometric derivatization provides high sensitivity and the described method gives reproducible results for 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 (40 pmol-1600 pmol).

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 brian tissue, thus disrupting all neuronal connections. Secondly, 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.

With the exception of the "identity of action" criterion, the most important criterion for establishing the identity of a neurotransmitter is demonstrating that stimulation of presynaptic axons evokes a synaptic release of the substance under study in sufficient quantity to activate the postsynaptic neuron. Classically, the stimulus-secretion coupling process is dependent on the presence of extracellular calcium ions (Rubin,1970).

A depolarizing concentration of K^+ (100 mM) in the artificial CSF selectively increased the rate of amino acid release into the perfusate. This concentration of K^+ was more preferable than that of

lower concentration (50 mM) since it provided consistent measurable release of the amino acids. The present study shows that appropriate depolarizing stimuli specifically alter the release of some physiologically active amino acids from the brain. The Ca^{2+} dependency of the release of amino acid is not inconsistent with this proposed role as neurotransmitters in the brain.

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).

Result from previous experiments in rats (Ito, Orlov and Shimoyama,1978) and rabbits (Ito, Nisimaru and Shibuki, 1979) suggest trophic involvement of the climbing fibers on P-cell performance. Thus, following olivary destruction with 3-AP in rats, inhibitory action of vermal P-cells on Deiters neurons declines rather rapidly, as suggested by reduced amplitude and delayed onset of the IPSP in Deiters neurons induced by

stimulation in the vermis. In rabbits, reflex discharges in vestibulospinal tract cells become drastically enhanced following electrolytic destruction of the caudal part of dorsal accessory olive, suggesting reduced reflex inhibition from the P-cells outflow. Few possibilities with regards the mechanism underlying the mentioned observations can be proposed. Firstly, such changes in synaptic efficacy of the P-cells may reflect decline in post- synaptic membrane responsiveness in the target neurons. Secondly, it is also possible that changes occur in the presynaptic compartment leading to reduced synaptic discharge of GABA at P-cell terminals.

Preliminary experiments by Ito, Sakurai and Tongroach (unpublished observation) , however, demonstrated that there was no changes in sensitivity of Deiters neurons to iontophoretically applied GABA following olivary destruction, suggesting no changes in sensitivity of GABA receptors on the target neurons of P-cells. This excludes the first possibility mentioned above.

The aims of the present experiment are to test the possibility of presynaptic involvement of the changes in P-cell performance following climbing fiber

denervation. It is proposed that such involvement may be suggested by the amount of evoked GABA release in the P-cell target areas.

With reference to GABA, stimulation of the P-cell terminals with depolarizzing concentration of K (100 mM) by perfusing the vestibular nuclei complex faiied to evoke the release in 3-AP lesioned rats (Fig. 16). This result alone seems compatible with the suggestion that deafferentation of P-cells climbing fibers leads to reduction of GABA release from the P-cell terminals. This would account for the reduction of P-cells synaptic efficacy observed by Ito, Orlov and Shimoyama (1978) and Ito, Nisimaru and Shibuki (1979). What causes the reduction in GABA release is still a matter of future study. Ito (1984) suggests that, since inactivation of olivary neurons by tetrodotoxin does not produce such attenuation of P-cell inhibition (Ito, Nisimaru and Shibuki, 1979), the effect of olivary destruction appears to be conveyed to axon terminals of P-cells independently of electrical process, presumbly by axoplasmic flow. If this is really the case, it may therefore be further suggested that disturbance of axoplasmic flow may prevent the supply of GABA synthesizing emzyme glutamic acid decarboxylase (GAD) from the P-cell body to its axon

terminals. This may in turn lead to reduction in GABA synthesis in the axon terminals.

In addition to reduction in GABA release, it is also observed in the present experiment that release of all other amino acids, namely : asp, glu, ser, glu-NH_2, gly, tau and ala was also reduced to the same extent as that GABA. This may seems rather unexpected, by one explanation can be drawn to address such result, which is the possibility that the P-cells axoplasmic flow may be greatly disturbed by climbing fiber deafferentation as suggested by Ito (1984). Such disturbance may lead to reduction of macromolecules entering the P-cells terminals indicriminatively.