CHAPTER I

INTRODUCTION AND BACKGROUND INFORMATION

Cerebellum consists of an outer mantle of gray matter, the cerebellar cortex, white matter and four pairs of deep nuclei namely, the fastigial, globose, emboliform and dentate nuclei. These nuclei are embedded in the white matter in medial to lateral order.

The cerebellum recieves input from the periphery and from all levels of the central nervous system. Most afferent fibers to the cerebellum synapse on neurons in these nuclei. Similarly, the outflows from most regions of the cerebellar cortex project first to the deep nuclei rather than directly out of the cerebellum excepts projections from the flocculonodular lobe which project directly to the vestibular nuclei in the brainstem.

General Background of the Fastigial Nucleus

The fastigial nucleus (FN) is the most medial nucleus of the deep cerebellar nuclei. It lies in the roof of the fourth ventricle and its size is second to the dentate nucleus. The irregular contours of the nucleus give it a variety of shapes in different planes of section, however, the main cell mass has an ellipsoidal configuration with the long axis oriented rostrocaudally (Carpenter and Batton, 1982). In the monkey, there are no recognized subdivisions in the nucleus but clusters of cells appear to form subgroups (Walberg and Jansen, 1961). The fastigial nucleus is characterized by a population of densely packed cells of varying size. Cells in the dorsal two-thirds of the nucleus are large and medium-sized, while small and very small cells

predominate in ventral regions. Large and medium-sized cells are polygonal, stain deeply with cresyl violet and contain coarse clumps of chromatin. Small cells are round or fusiform, rather pale and have fine, evenly distributed chromatin. The very smallest cells lie in the roof of the fourth ventricle and it is not clear whether they are neurons or glial cells. Cells is the FN of the monkey range in size from 10 to 65 μm with the greatest number of cells in the range between 17 and 35 μm (Courville and Cooper, 1970). Golgi studies of the rat fastigial nucleus indicate that both large and medium-sized cells have dendrites radiating in all direction that bear spines which are most numerous on distal branches (Matsushita and Hosoya, 1978). Common tree shrew FN is also ellipsoidal in shape and 1,200 μm long in rostrocaudal direction. The rostral pole of the nucleus is at the level p 3.2 (Luk-in, 1992). The largest part of the nucleus (approximately 50 μm in width) is at the level of the rostral part of the rostral of the open medulla. The nucleus is then gradually smaller caudally until the caudal pole was observed at the level p. 4.4 (Luk-in, 1992).

There is extensive overlap of dendritic fields in the nucleus. Axon of fastigial neurons project ventromedial and ventrolaterally, those passing ventrolateral appear to project to the vestibular nuclei, while those passing ventromedially cross to the opposite side presumably enter the uncinate fasciculus (UF). Approximately equally number of cells give rise to crossed and uncrossed axons, but axon crossing to the opposite side appear most numerous in rostral regions of the nucleus. No commissural fibers appear to interconnect the fastigial nuclei.

Cardiovascular Response Evoked by Fastigial Nucleus Stimulation

It has long been known that the cerebellum may influence blood pressure

and other cardiovascular functions. Focal electrical stimulation of the cerebellar cortex (Moruzzi, 1940 ; Zanchetti and Zoccolini, 1954 ; Hoffer, Ratcheson and Snider, 1966) or less commonly of the deep cerebellar nuclei (Zanchetti and Zoccolini, 1954 ; Ramu and Bergmann, 1967) or white matter (Sawyer, Hilliard and Ban, 1961 ; Ramu and Bergmann, 1967) has evoked changes in arterial blood pressure (ABP), alteration in heart rate (HR) and rhythm. It was later found that electrical stimulation within the ventromedial portion of the rostral third of the fastigial nucleus in the cat produced a large and abrupt rise in ABP and HR (Miura and Reis, 1969, 1970). The authors have termed it as the fastigial pressure response (FPR). The blood pressure increase consists of both systolic (SP) and diastolic (DP) which is as much as 100 mmHg each. The latency of the response is less than 2 s and it rapidly falls after cessation of the stimulus. Also, tachycardia is an associated phenomenon which may revert to a bradycardia after stimulation stop. The threshold for the response is quite low, averaging 0.05 mA, and graded. Several lines of evidences indicated that the FPR results from stimulation within the FN and is not due to spread of the current to the underlying brainstem, (a) with stimulus current at or less than 5 times threshold the response disappears as the electrode penetrate below or infront of the FN, (b) following a small electrolytic lesion placed at a positive locus in the nucleus abolishes the response, (c) the pattern of FPR response is different from that elicited by electrical stimulation of underlying brainstem, (d) the FPR elicited by stimulus frequencies within a relatively narrow band width of 30-80 c/s. This is contrast to pressor response from the underlying brainstem which are evoke at much wider frequency ranging between 80-300 c/s.

The FPR is abolished by bilateral lesion of the restiform body (RB), the intramedullary trajectory of the fastigiobulbar tract adjacent to the lateral vestibular nucleus (VL) and of the paramedian reticular nucleus (PRN) of the medulla (Miura and Reis, 1969, 1970). The authors concluded that the FPR is an unrecognized cerebellobuibar reflex acting on central blood pressure - modulating mechanisms, its principle relay centre to spinal cord is PRN.

Achari and Downman (1970) confirmed the previous finding in the cat after electrical stimulation in an unspecified position of the FN. They also reported electrodermal responses of the paws and pupil dilation with retraction of the nictitating membrane, and suggested that the fastigial stimulation causes sympathetic discharge to the heart and peripheral vessels due to the blocking effect of sympathetic drug.

Blood pressure response evoked by electrical stimulation of FN and carotid sinus nerve (CSN) were mutually inhibitory and summed algebraically (Miura and Reis, 1971). Interneurons are also identified in the PRN which are excited by axon terminals projected from ventromedial portion of the rostral FN or CSN neurons through polysynapses. Thus the authors concluded that there is a mutually inhibitory interaction between projections from FN and the CSN acting on vasomotor neurons.

Studies in the anaesthetized rabbit confirmed the pressor action of the rostral fastigial nucleus (rFN) and revealed concomitant enhancement of the renal sympathetic nerve discharge. In addition, the caudo-medial region of the nucleus was also shown as another effective site. The response evoked by this site however, exhibited a characteristic rhythmic fluctuation (Nishimaru and Kawaguchi, 1984 ; Bradley et al., 1987). These results suggest the cardiovascular responses may indeed be evoked from both the caudal and rostral FN, and these appear to be mediated by two distinct pathways (Bradley et al., 1987).

Electrical stimulation restricted to the rostromedial pole of the cerebellar FN in rat as in other species also elicit an elevation of arterial pressure and heart rate (Nakai, Iadecola and Reis, 1982 ; Del Bo, Sved and Reis, 1983).

The studies also extend to other parameter such as global elevation in cerebral blood flow (Nakai, Iadecola and Reis, 1982) elevation in plasma level of adrenal catecholamines vasopressin (Del Bo, Sved and Reis, 1983). However, the FPR may be due, at least in part, to stimulation of axons of passage since after bilateral destruction of perikarya in the FN with cytotoxic agent, kainic acid (0.5 mg) did not alter the blood pressure increased observed during monopolar electrical stimulation (Henry and Conner, 1989). Also, after chemical stimulation of FN perikarya with kainic acid (0.005-5 nmol) only elicited a sustained dose-dependent decrease in ABP and HR (Chida et al., 1986). Later, Chida, Iadecola and Reis (1990) concluded after electrical and chemical stimulation of FN perikarya in the rostral portion that ; (a) the FPR results from excitation and the fastigial depressor response (FDR) inhibition of reticulospinal sympathoexcitatory axons of the rostral ventrolateral reticular nucleus (rVL or C_1 area), (b) the FPR is a consequence of excitation of axons arising from neurons in an as yet unidentified area of lower brainstem project to or through FN and with collateral branches innervating, rVL mono or polysynaptically, (c) the FDR is contrast representing excitation of intrinsic neurons with a polysynaptic projection to rVL through unidentified regions of lower brainstem, (d) the rVL plays a critical role in intregrating action of the systemic and cerebral circulation represented in cerebellum.

The cardiovascular responses to electrical stimulation of FN have been described in both conscious and anaesthetized dogs (Dormer et al., 1986 ; Dormer, Person and Andrezik, 1989). The tachycardia portion of the FPR is uniquely buffered by the baroreceptor reflexes, however, the inotropic and pressor responses are sustained during stimulation. Thus, the increase in ABP is due to both increased cardiac output and peripheral vasoconstriction. A_5 area, dorsal and lateral to the facial motor nucleus, was found to be the prominent pressor relay area in the brainstem for the FN stimulation. Since the FN-induced in cardiovascular responses were reduced or abolished by direct current (DC) or radiofrequency or chemical (kainic acid) lesion while

lesions in the PRN, rostral and caudal to obex, failed to reduced the FN cardiovascular responses (Dormer et al., 1986). Lesion of nucleus of the solitary tract augmented the FPR and tachycardia while kainic acid lesion in A_5 area on the contrary reduced the FPR following electrical stimulation. Moreover, the rostral ventrolateral medulla (rVLM) which is associated with the C_1 catecholamine containing cell group of Hokfelt et al. (1974) was also found to be the brainstem vasomotor region as previously described in rat (Ross et al., 1983, 1984), cat (Mcallen, 1985), rabbit (Blessing et al., 1981 ; Dampney et al., 1982). Electrical stimulation in specific area of the FN in common tree shrew revealed the highest FPR response in 550 μm. The response is then decreased caudally, high response in 430 μm, medium response in 210 μm and no response in the area 560 um of the nucleus (Luk-in, 1992).

The FPR, HR and respiratory rate increase after electrical stimulation in the FN was confirmed in crab eating monkey and rhesus monkey. Only local mechanism stimulation during operation in the vicinity of the FN of the cerebellum elicit a FPR in a 6 year old child cerebellar tumor (Elisevich and Redekop, 1991). Summary of the previous finding of FN influence on cardiovascular function is shown in table 1.

Fastigial Efferent Projections

It is generally accepted on the basis of degeneration studies that cells of the FN give rise to two distinct efferent bundles. The first one is uncrossed bundle that emerge from the cerebellum via the juxtarestiform body (JRB) and the second is crossed bundle that forms the uncinate fasciculus (UF) (Carpenter, 1959). Uncrossed fastigial efferent fibers in the JRB are considered to arise largely from cells in the rostral portion of the nucleus, white fibers of the UF are regarded as arising

Table 1 Summary of the previous findings of FN influence on cardiovascular function

No.	Experimental Procedure	Results	Animal	Refferences
1.	Electrical stimulation of rFN.	Produced pressure response	Cat	Achari and Downman (1969, 1970, Achari, Al-Ubaiby and Downman (1973), Lutherer and Williams (1986), Bradley et al. (1987), Huang, Peng and Shich (1989), Williams et al., (1989)
			Dog	Dormer and Stone (1976), Dormer, Forman and Stone (1977), Dormer, Forman and Ohato (1982), Dormer et al., (1986)
			Rat	Del Bo, Sved and Reis (1983), Del Bo et al., (1983)

No.	Experimental Procedure	Results	Animal	Refferences
			Rabbit	Bradley, Paton and Spyer (1983), Bradley et al., (1987)
			tree shrew	Luk-in, (1992)
			Monkey	Sudsuang et al., 1990
2.	Mechanical stimulation in vicinity of FN	Produced pressure response	Human	Elisevich and Redekop (1991)
3.	Electrical stimulation of rFN.	Inhibited a baroreceptor -evoked bradycardia	Cat	Achari and Downmar (1970)
4.	Electrical stimulation of rFN.	Inhibited BP response of carotid sinus stimulation	Cat	Miura and Reis (1971)
5.	Electrical stimulation of rFN.	Excited spinal sympathetic nerve activity	Dog	Dormer, Foreman and Ohato (1982)

No.	Experimental Procedure	Results	Animal	Refferences
6.	Electrical stimulation of of rFN.	Released renin	Cat	Kayama, Ammons and Manning (1980)
7.	Electrical stimulation of rFN.	Released adrenomedullary catecholamines	Rat	Del Bo et al., (1983)
8.	Electrical stimulation of rFN.	Released vasopressin	Rat	Del Bo, Sved and Reis (1983, 1984a, 1984b)
9.	Electrical stimulation	Excited renal sympathetic nerve activity	Rabbit	Nishimaru and Kawagushi (1984)
10.	Fastigial lesion	Impaired the tilting response of BP and HR	Cat	Miura and Reis (1970)
11.	Fastigial lesion	Abated cardiodynamic pattern during compensatory phase of the orthostatic reflex	Cat	Kayama, Ammons and Manning (1981)
12.	Fastigial lesion	Decreased in ABP and HR during exercise	Dog	Dormer and Stone (1982),Dormer (1984)

No.	Experimental Procedure	Results	Animal	Refferences
13.	Fastigial lesion	Prevented the recovery of BP after hemorrhage	Dog	Lutherer et al., (1983), Lutherer, Williams and Everse (1989)
14.	Fastigial lesion	Attenuated vasopressin release in response to hemorrhage	Rat	Sved, Scott and Kole (1985)
15.	Chemical stimulation of FN.	Glutamate produced a slowly evoluing hypertension with bradycardia	Dog	Dormer, Foreman and Stone (1977)
16.	Chemical stimulation of FN.	Kainic acid induced a depressor response	Rat	Chida et al., (1986)
17.	Chemical stimulation of FN.	Glutamate induced a depressor response	Cat	Bradley et al., (1987)

predominantly from cells in the caudal part (Cohen, Chambers and Sprague, 1958 ; Carpenter, 1959 ; Wallberg et al., 1962 ; Angaut and Bowsher, 1970). However, the degeneration techniques also interupt fibers traversing various portions of the nucleus. Thus, this is difficult to interpret the pattern of results. Observations based upon anterograde transport of (H^3) amino acid suggest a different origin of the fibers in these bundles (Carpenter and Batton, 1982). Fairly selective unilateral labelling of cells in either the rostral or caudal parts of the FN resulted in transport of the isotope via uncrossed fibers in the JRB and crossed fibers in the contralateral UF. Although transport of isotope via these bundles was not as great as when the entire FN was labeled, these data suggested that cells at all rostrocaudal levels of the nucleus give rise to crossed fibers, while axonal collaterals or other cells project fibers ipsilaterally into the brainstem, the crossed fibers seem greatly exceed those that are uncrossed. Efferent projection from the FN to brainstem nuclei and intermediolateral (IML) cell column in thoracic spinal cord through those crossed and uncrossed tracts have been revealed by degenerative, anterograde transport of (H^3) amino acid, retrograde horseradish peroxidase (HRP) and wheat germ agglutinin - horseradish peroxidase (WGA-HRP) anterograde and retrograde technique. Summary of the connections will be described as follow :

1. Fastigiovestibular Projections

Data from degeneration studies suggest that efferent fibers project bilaterally and differentially to parts of the four vestibular nuclei and to certain paravestibular nuclei (Carpenter, 1959 ; Thomas et al., 1956 ; Walberg et al., 1962).

In rhesus monkey, the unilateral lesion of the FN produces bilateral degeneration in the UF and ipsilateral degeneration of fibers in the JRB. The

contralateral UF fibers are predominantly originated from the caudal portion of the FN while most of those fibers of the ipsilateral UF are originate mainly from the rostral portion of the FN. The uncinate fibers terminate in all vestibular nuclei, the greatest number pass to and through the lateral and descending vestibular nuclei. Most fibers in the JRB appear to terminate only in lateral and descending nuclei (Carpenter, 1959). Evidences in the cat reveal minor differences from those rhesus monkey, the contralateral UF fiber also terminate in the small cell groups "f" and "x" of Brodal and Pompeiano (1957).

The terminations of the ipsilateral fibers are restricted to certain regions within each of the four nuclei (Walberg et al., 1962). Particular significance was attached to the somatotopical projections to the lateral nuclei of both sides. Those crossed fibers are described as projecting to ventral region of the nucleus while uncrossed fibers were said to terminate in dorsal region. Because the cerebellar vermis projects somatotopically in a regular fanlike manner upon the FN (Jansen and Brodal, 1940) and the vestibulospinal projection is somatotopically organized (Brodal and Pompeiano, 1957) thus, the hypothesis was advanced that a somatotopic linkage existed between the vermal cortex and the spinal cord which involved both the FN and VL (Walberg et al., 1962).

Crossed fibers are suggested to terminate in ventral region of the inferior vestibular nucleus (VI) while uncrossed fiber end in more dorsal region. Unlike other vestibular nuclei projections to the medial vestibular nuclei (VM) are reported to cover the entire nucleus ipsilaterally except for its ventral margin (Carpenter and Batton, 1982). Both crossed and uncrossed fibers chiefly terminate in the peripheral region of the superior vestibular nucleus (VS) (Walberg, 1958 ; Walberg et al., 1962). Similar results are observed in other mammalian species such as rat (Achenbach and Goodman, 1968) and common tree shrew (Ware, 1973).

Selective injection of (H^3) amino acid in rostral, caudal and all parts of the FN found that the transport of isotope primarily passed via fibers of the contralateral UF and the ipsilateral JRB (Batton et. al., 1977). Fastigial projections to vestibular nuclei are mainly to ventral portion of the VL and VI. They are nearly the symmetrical and quantitively similar on each side. Those project cell group "f" and "x" arise from all parts of the FN and are mainly crossed. Modest projections to the VM are uncrossed. No fastigial efferent fibers terminate in SVN on either side or in dorsal region of the VL. Cross fibers terminate in VI. The most notable difference between the two sides concerned the asymmetrical fastigial projection to ; a) cell group "f", b) cell of "x" and c) the VM (Moolenaar and Rucker, 1976 ; Batton et al., 1977).

Fastigovestibular projections are confirmed in cat and squirrel monkey by retrograde transport of HRP or mixture of HRP and WGA-HRP after microinjection in the vicinity of VM, VI and VL (Carleton and Carpenter, 1983). It was found that, neurons in all part of the FN project contralaterally to the VM ; those in ventrolateral in the central third of the nucleus, with contralateral dominance project to VI. Neurons in the rostral parts of the nucleus project bilaterally to the VL.

2. Fastigioreticular Projections

Fastigial efferent fibers projecting to the reticular formation of the pons and medulla have been described by serveral authors using variety of degeneration techniques. Study in the rhesus monkey presented fibers of the descending component of the UF project to the paramedian area of the reticular formation of the pons and medulla from the level of the abducens nuclei to the level of the hypoglossal nuclei (NH) (Carpenter, 1959).

Earlier degenerative studies have shown efferent connections of the cerebellar nuclei to the reticular formation of the brainstem (Allen, 1927). Such fibers have been described in more details by a few groups of investigators (Rand, 1954 ; Thomas et al., 1956 ; Carpenter, Britten and Pines, 1958 ; Cohen, Chambers and Spraque, 1958). However, none of these studies suggested a topical arrangement within the fastigioreticular projections, i.e. whether fibers from certian parts of the FN are distributed to particular regions of the reticular formation.

Following total or partial lesions of the FN in cat (Walberg et al., 1962) the ensuring degeneration in medullary and pontine reticular formation have been studies in silver impregnated section by method of Nauta and Glees. It was found that, all parts of the FN appear to project mainly contralaterally to the medial two-third of the reticular formation, while only small amount of the ipsilateral fiber project to the area. Most of the fibers terminate in the nucleus reticularis gigantocellularis (NRG), nucleus reticularis tegmenti pontis (NRT) and the PRN. Only modest degeneration is present in the nucleus reticularis pontis oralis and caudalis, nucleus reticularis ventralis, nucleus reticularis parvicellularis, nucleus subcoeruleus and nucleus reticularis laterals. However, no orderly arrangement has been found in the fastigioreticular connection. This finding is different from what has been found for the fastigiovestibular projection.

Neurons in the caudal half of the rat was demonstrated to project contralaterally and form the hook bundle of russel passing the lateral and inferior vestibular nuclei en route to the reticular formation (Achanbach and Goodman, 1968) distributing among cells of the medial half of the reticular formation. These degenerating axons were present the region thoughout the entire caudal extent of medulla ; particularly in NRG and nucleus reticularis pontis caudalis. At pontine and

rostral medullary level, degenerating axons occurred in modest number in the lateral reticular formation particularly in nucleus reticularis parvocellularis, connections to these lateral regions decrease in number at mid-medullary levels and are not present caudal to the level of the NH. However, this results donot seem to be in line with those in monkey which found fastigioreticular arise mainly from the rostral region of the FN. These fibers are entirely crossed and project mainly to medial regions of the NRG, dorsal PRN and the magnocellular part of the lateral reticular nucleus (RL) (Batton et al., 1977 ; Carpenter and Batton, 1982). Results obtained in the cat after injection of the rostral FN revealed the harviest deposition of silver granules in medullary reticular formation, paramedian and gigantocellular divisions (Moolenaar and Rucker, 1976). Projections from ventral and lateral borders of the FN project contralaterally to the ventral portion of the RL after studies in cat using retrograde transport techniques of WGA-HRP. In rat, however the FN project contralaterally to the dorsal part of the middle third of the RL (Rajakumar, Hrycyshyn and Flumerfelt, 1992).

3. Fastigiospinal Projections

In the late nineteenth century several studies on the dog and cat reported that fibers originating in the cerebellum terminated in spinal cord through at least lumber level (Marchi, 1891 ; Mingazzini, 1894 ; Thomas, 1897). This tract was described as exiting from the cerebellum as part of the descending limb of the brachium conjunctivum (BC), in the spinal cord these cerebellospinal projections were observed in the anterolateral column. More recent experimental studies utilizing modern stain techniques were not able to substantiate the earlier reports of an extensive cerebellospinal tract. Degenerative studies using Marchi staining technique, Gray (1926) and Rasmussen (1933) in the cat and Foltz and Matzke (1960) in the opossum described a small cerebellospinal portion of the hook bundle of Russel terminating in upper cervical levels, whereas the caudal most cerebellar projection that

Carpenter, Britten and Pines (1958) could trace ended at the level of the NH. In the monkey, Carpenter (1959), Carpenter and Nova (1960) could not follow cerebellofugal fibers caudal to the medulla. With the Nauta-Gygax method, Thomas et al. (1956) in the cat and Mehler (1967) in the rat traced a small amount of hook bundle degeneration into upper cervical spinal regions following lesion in the FN. This hook bundle projection to the spinal cord should not be confused with data in the recent literature concerning the contralateral ventral descending limb of the BC which has not been traced caudal to mid-medullary level (Achenbach and Goodman, 1968). Achenbach and Goodman, (1968) presented different results demonstrating that the FN projects fiber via the hook bundle and appear to pass ventrally to terminate in intermediate gray and lamina VII of ventral horn gray. These fibers could be traced through lumbar levels with the amount of degeneration was greatest in cervical regions and decreased in quantity significantly in thoracic and lumbar levels (Achenbach and Goodman, 1968). Similar results were obtain from study in the common tree shrew (Ware and Mufson, 1979).

An autoradiographic study, after injection (H^3) amino acid (L-Leucine, L-proline, and L-lysine) into the rFN, reported projection from the rostral portion of the nucleus to the anterior gray horn at C_1 (Batton et. al., 1977). However, this data could not be assured due to the fact that the authors could trace the terminals in two monkeys out of five experimental ones. With more precise HRP retrograde transport technique, Fukushima et al. (1977) could reveal direct fastigiospinal pathways in cat with cells to C_2 C_3 cervical level of origin of this pathway are located mainly in the rostral part of the contralateral FN. This data has been confirm by later investigators (Matsushita and Hosoya, 1978). However, contradicted results are found between the two groups of investigators, while Fukushima et al. (1977) demonstrated labelled cell throughout the contralateral nucleus, but were most numerous in the rostral parts, Matsushita and Hosoya (1978) reported that the distribution is primarily in the central and caudal parts of the FN. and

extended ventrolaterally into a zone between the fastigial and the anterior interposed nucleus.

At present, so far no literature demonstrates efferent projections from the precise FPR areas of the FN. to specific terminal areas in the brainstem and spinal cord. Previous investigators undertake two separate categories of experiments. One for those who explained efferent projections to various nuclei in the brainstem and spinal cord after degeneration injection of (H^3) amino acid or HRP or WGA-HRP in the FPR-assumed area of the FN. Which other stimulate areas of the FN. to demonstrate the FPR. Moreover, techniques employed in previous have limitations which labile for misinterpretations (Izzo, 1991).

This study is aimed to reveal efferent projections and their terminal nuclei in the brainstem and spinal cord from the FPR-area of the FN. This can be accomplished by simultaneous stimulation the various areas of the FN to show the FPR and injection of biocytin to reveal anterograde transport from the areas. By this approach we are quite confident to suggest that those efferent projections and their terminal areas originate from the FPR area. Moreover, this approach are still offer evidences for anterograde transport from various specific FPR area of the FN ; e.g. the rostral and middle portions.

Biocytin is a low molecular weight, soluble complex of biocytin and amino acid lysine (Fig 1) the biocytin has unique selectivity and high affinity with the glycoprotein avidin (Wright et. al., 1952). This property has been extensively exploited which allows for the sensitive detection of biocytin. More recently it has been suggested that the extracellular injection of biocytin results in the labelling of neurons at the injection site and their efferent axon at light microscopic (King et al.,

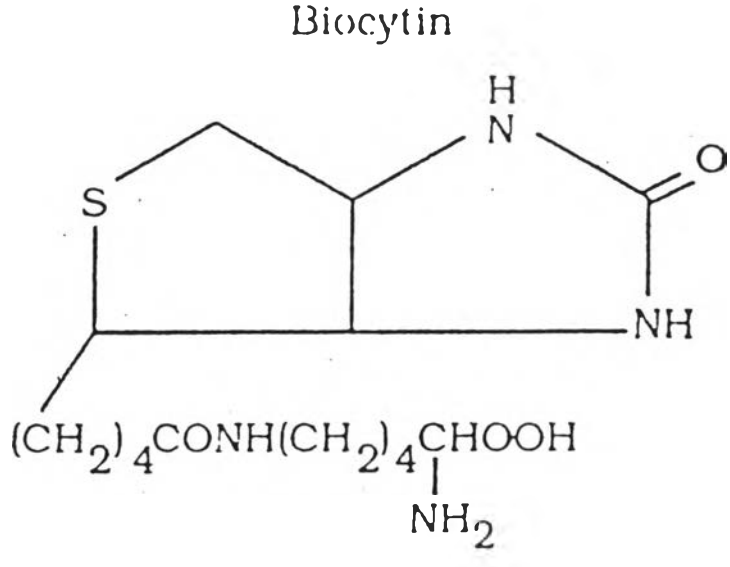

Fig 1 The structure of biocytin

1989) and ultrastructural levels (Izzo, 1991) allowing the examination of anterogradely labelled axon and their synaptic connections.