HISTOCHEMICAL AND ELECTROPHYSIOLOGICAL STUDIES ON THE ORIGINS AND SITES OF TERMINATION OF THE SPINAL SEROTONERGIC PATHWAY

A Thesis
Presented to the
University of Manitoba

In Partial Fulfillment of the Requirements
for the Degree

MASTER OF SCIENCE
IN
PHYSIOLOGY

by

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May, 1976

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ACKNOWLEDGEMENTS

I would like to take this opportunity to express my gratitude to different individuals whose advice and encouragement have been instrumental to me in the course of this thesis.

Firstly, I would like to express my thanks to my parents, Joseph and Margaret Menzies, for their confidence in me, and to my family, Leslie and Jordan, who were so patient in living with an absentee husband and father while I carried out this work. Sincere appreciation is extended to Dr. L.M. Jordan for the continued interest and support he has shown in both my academic and research education, for providing me with an example of thoroughness and dedication to science and in whose laboratory this work was carried out. Thanks go as well to Dr. Dave Humpherys for introducing me to research in the first place, as well as to fellow graduate students and friends with whom I spent many hours discussing my project. I would also like to thank the members of my committee, Drs. V. Havlicek, K.R. Hughes and C. Braekevelt for their critical analysis of this thesis and Donna Carroll who spent many hours typing the manuscript.

This work was made possible by a Medical Research Council of Canada grant to Dr. L.M. Jordan.

ABSTRACT

In an effort to determine the distribution of 5-HT terminals in the feline spinal cord, cats were injected intraspinally at the thoraco-lumbar junction with 6-hydroxydopamine in order to deplete spinal noradrenaline levels. The results of these experiments indicate that the majority of 5-HT terminals are located in the ventro-medial aspect of the ventral horn in Rexed's lamina VII, the dorso-medial part of lamina VIII, and lamina IX. Many terminals were also seen in the more ventro-lateral area of the ventral horn encompassing the lateral lamina IX, and in lamina X in the area of the central canal. Few terminals were found in lamina II and III in the dorsal horn, with very few in the intermediate zone.

In order to measure the conduction velocities of the 5-HT fibers projecting to the spinal cord, the antidromic responses to stimulation of the spinal cord at L_1 were made in the raphé nuclei of the caudal pons and upper medulla. The results revealed that the conduction velocities of bulbospinal serotonergic fibers ranged between 17.3 and 120 m/sec. Such values were far greater than those expected for small unmyelinated fibers (2.5 - 3.5 m/sec).

In order to explain the results, sagittal sections of thoracic spinal cord were examined in chronic spinal cats for fibers containing 5-HT. These sections were counterstained with Luxol Fast Blue and examined for myelin sheaths.

The results indicated that at least a portion of these fibers were surrounded by a myelin sheath, and may be capable of conducting at the observed conduction velocities. These results may indicate the presence of a fast bulbospinal serotonergic pathway.

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INTRODUCTION

Interest in the possibility that serotonin (5-hydroxy-tryptamine - 5-HT) may be a neurotransmitter in mammalian central nervous system was initially provoked by the discovery of its presence in the brain by Twarog and Page (1953) and Amin et al (1954). Since then numerous investigations have been concerned with mapping out the locations of 5-HT containing cell bodies and terminals in various parts of the brain and spinal cord. Using the then recently developed technique for the demonstration of fluorescent compounds of monoamines (Falck, 1962; Falck et al, 1962), Dahlstrom and Fuxe (1964) succeeded in demonstrating the presence of 5-HT containing cell bodies in the raphé nuclei of the lower brainstem of the rat. More recently it has been confirmed microspectrofluorimetrically (Jonsson et al, 1975) that the majority of cell bodies in these nuclei are capable of synthesizing, storing and metabolizing 5-HT. Such findings are consistent with a role for 5-HT as a CNS transmitter, showing that it fulfills many of the established criteria for transmitters (cf., Werman, 1966; Phillis, 1970).

Since the early demonstration of the presence of 5-HT in cells of the raphé nuclei, several investigators have attempted to delineate both biochemically and histochemically, the distribution of this monoamine in various other CNS regions. Fluorescence histochemical studies (Carlsson et al, 1962; Anden et al, 1965; 1966 a,b; Fuxe, 1965) have shown the presence of

5-HT in various diencephalic and telencephalic loci, where it is confined to small (.3 - 1.0 um) beadlike "varicosities" which, in many cases, were seen to make close contact with neuronal perikarya. These varicosities were presumed to be the terminals of 5-HT containing axons, and the localization of serotonin to such terminals strengthened the notion that it may be involved in transmission at serotonergic synapses. Further work by these investigators (Carlsson et al, 1964; Fuxe, 1965) also demonstrated similar tryptamine containing varicosities in the spinal cord of both mouse and rat, a finding confirmed by Anderson and Holgerson (1966). These studies have shown the largest numbers of these terminals to be located in the sympathetic lateral column and the ventrolateral and dorsolateral motor groups of the anterior horns in the cervical and lumbar enlargements. At these levels the presence of 5-HT containing varicosities in the medial motor group is less than in the corresponding motor group at thoracic levels. Scattered terminals are distributed throughout the dorsal parts of the posterior horn at all levels, with somewhat fewer of them in the remaining areas of this horn. In the intermediolateral column it appears that most of the nerve cells are surrounded by terminals containing 5-HT, and it has been reported that some of the 5-HT varicosities in the anterior horns of cervical and lumbar enlargements make "intimate contact" with dendrites and cell bodies of motoneurones. On the other hand the cells of the ventral area of the dorsal horn

seem to have fewer contacts with 5-HT terminals with even less cellular contact by terminals in the superficial aspects of the dorsal horn (Carlsson et al, 1964).

In addition, several biochemical investigations have confirmed and extended the findings referred to above. Anden (1964) showed that there is usually two to four times as much 5-HT as noradrenaline (NA) at all locations in the cord, with more in the gray matter of the lateral horns than the anterior and posterior horns and very low levels in the white matter. Recently, Zivin et al (1975) have confirmed this distribution for rabbit and rat spinal cords in a more precise fashion through the use of microdissection techniques, and have shown that a substantial amount of 5-HT may also be seen in the area of the central canal.

Of central importance in this discussion is the question of whether or not the 5-HT terminals observed by fluorescence microscopy originate from the cell bodies of the raphe shown to contain this amine. Correspondingly, investigations aimed at resolving this issue have dealt with the biochemical and histochemical distribution of 5-HT after lesions in the brain and spinal cord which are thought to interrupt monoamine pathways. Caudal to a transection in the mid-thoracic region of rabbits, 5-HT levels fell to a minimum after seven to nine days, while there was a slight rise in cord 5-HT content rostrally (Anden et al, 1964b).

Fluorescence histochemical studies (Carlsson et al, 1964; Dahlstrom and Fuxe, 1964; 1965) have also shown that there is a significant loss of monoamine fluorescence caudal to a spinal transection even after treatment with agents known to enhance visualization of fluorescent products. Rostral to the transection however, there is improved visibility of fluorescence due to accumulation of amines in the end of the axons above the transection, and it has also been reported that following administration of the monoamine oxidase inhibitor Nialamide (which improves fluorescence due only to 5-HT) it was possible to trace axons containing 5-HT from the site of the transection up to 5-HT containing neurone soma in the medulla oblongata of the rat (Dahlstrom and Fuxe, 1964).

In cross sections of these spinal cords above the transection it was possible to visualize a build-up of 5-HT in non-terminal axons and to discern their position of travel in the spinal cord. The fibers were seen to be very fine with small varicosities (Carlsson et al, 1964), while the sizes of the fibers themselves have been reported to be between 1 - 2 μm (Dahlstrom and Fuxe, 1965) and were unmyelinated. This latter suggestion is based on the findings of Dahlstrom and Fuxe, (1965) that fluorescent axons in "about the same location" as those showing a build-up of amine after transection of the cord did not show the weak brown fluorescence rings characteristic of

myelin. It has also been suggested that the failure of Heller et al (1966) to trace degeneration of nerve fibers to neocortex in cats after destruction of the median forebrain bundle is explainable because the 5-HT fibers ascending in this pathway are not myelinated and, hence, would not appear in Nauta stained material.

It was thus demonstrated that the descending fibers travel in the dorsolateral, lateral, and anterior funiculi near the exterior surface of the spinal cord and give off collaterals that enter the gray matter at all levels. This distribution within the white matter of 5-HT axons in the rat has also recently been confirmed for the cat by Coote and Macleod (1974).

After hemisection of the spinal cord, the reduction in fluorescent terminal density was less than after complete transection, and it could be seen that many terminals both contralateral as well as ipsilateral to the lesion remained. This is suggestive of crossed and uncrossed pathways from the raphe. (Carlsson, etal, 1963)

The findings presented thus far are strongly suggestive of a function of 5-HT as a neuro-transmitter originating from the brainstem and having both ascending and descending projections. Further support for this idea is provided by the findings that electrical stimulation of the mesencephalic

raphé nucleus in rats can cause release of 5-HT and 5-hydroxindol - 3 - ylacetic acid from cerebral cortex of rats (Eccleston et al, 1969), depletion of spinal cord 5-HT (Dahlstrom et al, 1965) and release of 5-HT but not acetylcholine (ACh) into the lateral ventricle of cats (Ashkenazi et al, 1972). In addition, Anden et al (1964a)have shown release of 5-HT into a perfusion solution of isolated frog and mouse spinal cords by electrical stimulation of the upper end of the cord, and Dahlstrom et al (1965) have demonstrated a decrease in both the number and intensity of fluorescent terminals remaining after 1 - 2 hours of tetanic electrical stimulation of the medullary raphé nuclei in rats.

So far the evidence is suggestive for 5-HT as a neurotransmitter in mammalian CNS,and although its exact role in the spinal cord has not yet been elucidated, it is thought to play a role in modulation of both motor and sensory functions at the spinal level.

Intravenous administration of the 5-HT precursor, 5-hydroxytryptophan (5-HTP) (100 mg./kg.),into cats spinalized at the first cervical level has been shown to increase the L_7 monosynaptic reflex (MSR) by up to 300 percent of control values (Anderson and Shibuya, 1966). Such an increase in the monosynaptic reflex was also accompanied by an increase in alpha motoneuronal discharge measured in ventral root filaments. Such findings were described earlier by Anden et al (1964c) and

Lundberg (1965). In addition to these findings, Anden et al and Lundberg have shown that intravenous 5-HTP in acute spinal cats causes marked depression of transmission in the pathway from flexor reflex afferents (FRA) to alpha motoneurones. After administration of a decarboxylase inhibitor there is no effect from large doses of 5-HTP, and this has led to the conclusion that 5-HTP works entirely through formation and liberation of 5-HT from nerve terminals (Anden et al, 1964c).

After administration of monoamine oxidase inhibitors, the MSR height was selectively increased, whereas there was no significant change in the polysynaptic potentials (Anderson et al, 1967). This increase in MSR could not be prevented by the use of the peripheral noradrenergic alpha receptor blocker phenoxybenzamine, although the facilitation of the MSR due to intravenous administration of the NA precursor, L-3, 4 - dihydroxyphenylalanine, was effectively antagonized by phenoxybenzamine. Banna and Anderson (1968), however, have shown that cinanserin, methysergide and d-lysergic acid diethylamide (LSD-25) can block the 5-HTP-induced facilitation of the MSR without altering the inhibitory effects of 5-HTP on polysynaptic reflexes, indicating that the facilitatory, but not the inhibitory, effects of 5-HTP on spinal reflexes may be mediated by serotonin released from nerve terminals of a descending pathway.

In addition to the effects on alpha motoneurones, there has also been shown an effect of intravenous 5-HTP on

gamma (γ) motoneuronal discharge. Dixon et al (1969) have shown that the discharge of single fusimotor fibers to semitendinosus muscles in decerebrate spinal rabbits is made "regular" after i.v. 5-HTP or LSD-25, but such effects are not seen in chronic spinal rabbits. Further to this, Ahlman et al (1971) have shown the occurrence of a tonic stretch reflex after 5-HTP, probably mediated by the concomittant increase in the discharge rate of static gamma motoneurones. Later studies by Ellaway et al (1973) and Ellaway and Trott (1975) disagree with the interpretation of Ahlman et al regarding the basis for the facilitation of the tonic stretch reflex. They did confirm, however, the finding that gamma motoneurone discharge is increased after 5-HTP.

Such findings are of considerable interest in view of the role of 5-HT in locomotor behavior of rabbits. Viala and Buser (1969, 1971, 1974; Viala et al, 1974) have shown that 5-HTP administration in acute spinal rabbits can cause rhythmic flexion and extension of the hind limbs, an action reminiscent of that of L-DOPA in acute spinal cats (Anden et al, 1964c). While 5-HT does not cause such obvious effects in acute spinal cats, Anderson and Shibuya (1966) have reported "integrated flexion and extension" and exaggerated flexion reflexes after 5-HTP administration in cats spinalized at the first cervical levels, although they made no attempt to study this finding

directly. The results are nevertheless highly suggestive of a possible involvement of 5-HT in mammalian locomotor behavior.

Because there is much evidence for a descending tryptaminergic pathway, but only scant reason to suspect segmental 5-HT containing neurons (Bjorklund et al, 1970), several investigations have employed electrical stimulation of the raphé nuclei to activate the terminals of this pathway in the spinal cord. Thus, several studies were able to demonstrate either bulbospinal inhibition (Clineschmidt and Anderson, 1969) or a time dependent facilitation and depression of the segmental MSR (Clineschmidt and Anderson, 1970; Proudfit and Anderson, 1973). Because the bulbospinal inhibitory effects were antagonized by various 5-HT antagonists, it was concluded that a 5-HT neurone was interposed in the bulbospinal inhibitory pathway (Clineschmidt and Anderson, 1970). If such a view were correct, then an excess of 5-HT at the synaptic terminal should potentiate the effects of stimulation of the raphé nuclei. Sinclair and Sastry (1974 a,b), however, have shown that bulbospinal inhibition is blocked, not enhanced, by imipramine, desipramine and pargyline, drugs shown to prevent re-uptake of 5-HT, and on the basis of these results they suggested a tryptaminergic pathway that exerted inhibitory control over the bulbospinal inhibitory pathway, the locus of this control being uncertain (Sinclair and Sastry, 1974a). In addition, these authors have suggested that a serotonergic pathway may enhance the NA mediated tonic inhibition

of the recurrent inhibition of quadriceps monosynaptic reflex (Sinclair and Sastry, 1974b). Such a proposal is, therefore, not in agreement with the earlier work of Clineschmidt and Anderson (1970).

In addition to the involvement of 5-HT in spinal reflexes, recent interest has focused on a possible involvement in pain, and several investigators have assigned a role for 5-HT in analgesia and the effects of morphine. It was initially shown by Liebeskind et al (1973) that analgesia to peripheral noxious stimuli could be produced by stimulation of the periaqueductal gray matter in cats. Later investigations showed that the evoked firing of certain lamina V interneurones to a peripheral noxious stimulus was depressed by electrical stimulation of the raphe nuclei (Oliveras et al,1974) and a suppression of this inhibitory effect could be produced by LSD-25 (Guilbaud et al, 1973). Samanin and Valzelli (1971) concluded that dorsal raphe stimulation could increase the effectiveness of a sub-analgesic dose of morphine, while Sinclair (1973) suggested a blockade of bulbospinal inhibition of the MSR by i.v. morphine. At a cellular level, Randic and Yu (1975) have described depression of nociceptive cells in dorsal horn lamina I neurones following iontophoretically administered 5-HT. Such a finding is somewhat surprising, however, since this area has been shown to contain very few 5-HT terminals (Carlsson et al, 1964; Fuxe, 1964; Dahlstrom and Fuxe, 1965). Proudfit and Anderson (1973) have

suggested a reduction in the dorsal root potential in Group II and Group III afferents after stimulation of the raphé nuclei, an interesting finding in view of the fact that some of Group III afferents may carry "pain information" (Iggo, 1959, 1960).

In addition to the studies employing administration of 5-HT precursors or antagonists, or electrical stimulation of the brainstem to investigate 5-HT effects in the spinal cord, other studies have investigated the effects of 5-HT administered to single spinal neurones by microiontophoresis (Curtis, 1964). In barbiturate anaesthetized cats, Curtis et al (1961) failed to find any spinal neurones sensitive to 5-HT, but such results are different from those obtained in unanaesthetized preparations. Engberg and Ryall (1966) examined the effects of 5-HT on spinal alpha-motoneurones, Renshaw cells, and interneurones of cat ventral horn, and showed that 5-HT reduced the firing of 13 of 48 interneurones excited by D, L-homocysteic acid (DLH), but was without effect on motoneurones or the remaining interneurones. This lack of effect on motoneurones is somewhat surprising in view of the increase in motoneuronal discharge seen after i.v. infusion of 5-HTP or l-tryptophan (Anderson and Shibuya, 1966). 5-HT did, however, reduce the acetylcholine (ACh) or DLH-induced firing of some Renshaw cells, a finding later confirmed by Jordan and McCrea (1976). Weight and Salmoiraghi (1966) further showed that cat