Fonnum

Amino Acids as Chemical Transmitters

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PREFACE

This volume represents the proceedings of a NATO Advanced Study Institute on Amino Acids as Chemical Transmitters, which took place at Spåtind Hotel in Norway, August 14-21, 1977. The meeting is related to two previous meetings on metabolic compartmentation in the brain. The first of these meetings took place at Rockefeller Foundation, Bellagio, Italy, July 11-16, 1971 and the proceedings, Metabolic Compartmentation in Brain, were edited by R. Balazs and J.E. Cremer and published by Macmillan in 1973. The second meeting was an Advanced Study Institute on Metabolic Compartmentation and Neurotransmission Relation to Brain Structure and Function, which was held in Oxford, September 1-8, 1974. The proceedings were edited by S. Berl, D.D. Clarke and D. Schneider and published as Volume 6 of the NATO ASI Life Science series by Plenum Press.

The object of the present meeting was to review and discuss the present status of amino acids as chemical transmitters. Several issues such as electrophysiological response, localization, synthesis, release and receptor binding of transmitter candidates were discussed. The possible morphological correlates to these functions were also reviewed. During the meeting 24 leading papers were given. In addition, several of the participants presented important new findings during the discussion. Some of these have been included as short reports.

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F. Fonnum
Oslo, October 1977

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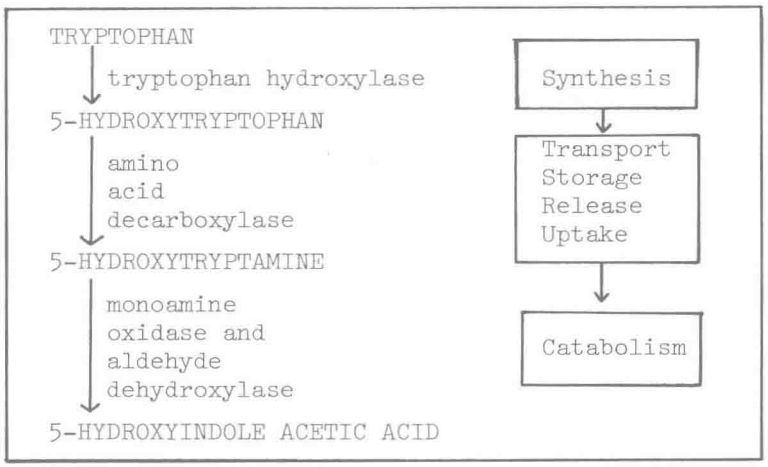
The aim of this report is to gather available evidence from the literature and to provide fresh evidence from new experiments for the morphological intraneuronal correlates for transmitter synthesis, packaging, transport, uptake, and metabolism in the mammalian central nervous system. A large body of literature is available for the catecholamines (norepinephrine and dopamine) and gamma-amino butyric acid and they have been the subject of recent extensive reviews (Bloom, 1973; Roberts et al., 1976). The present report will explore the indoleamine-serotonin systems. The pathways for the biosynthesis and catabolism of serotonin (5HT) and related indoleamines are well known and aptly discussed by Cooper et al., 1974, pp. 175-199. The essential steps are summarized in Table 1. The essential amino acid tryptophan is converted enzymatically by tryptophan hydroxylase, the rate limiting enzyme, to 5-hydroxytryptophan which undergoes decarboxylation to form 5-hydroxytryptamine or serotonin (5HT). These initial steps represent the synthesis of 5HT; once formed, 5HT is stored, transported or released by the indoleamine neuron in the course of its activity. 5HT is metabolized by monoamine oxidases in the presence of aldehyde dehydrogenase to form 5-hydroxyindole acetic acid. This report will review the findings of several pertinent studies using cytochemical or immunological methods in order to discover the intracellular organelles in indoleamine neurons which relate to the synthesis, transport, uptake, and catabolism of 5HT.

The initial approach for studying 5HT neurons at the cellular level has been through the Falck-Hillarp fluorescence technique. Dahlström and Fuxe (1964) pioneered the mapping of 5HT neuronal cell bodies in the brain stem by this method, after pretreatment of

TABLE 1. PATHWAYS IN SYNTHESIS AND CATABOLISM OF 5HT



the animals with monoamine oxidase inhibitors. These authors referred to the groups of yellow, transiently fluorescent neurons as groups B_1 to B_9, roughly divided as follows: B_1, the nucleus raphe pallidus and neighboring cells groups; B_2, the raphe obscurus; B_3, the raphe magnus, nucleus paragigantocellularis lateralis and a few other cells; B_4, the area postrema; B_5, the raphe pontis and other neurons; B_6, neurons beneath the floor of the fourth ventricle; B_7, raphe dorsalis and the caudal portion of the Edinger-Westphal nucleus; B_8, the median raphe, raphe linearis and neurons in the neighboring reticular formation; B_9, the neurons within and around the medial lemniscus. The major problem with the fluorescence methods for 5HT is their insensitivity for the display of most terminal axonal fields. Nevertheless, the method remains a potent tool for revelation of neuronal cell bodies that contain endogenous 5HT. Numerous attempts have been made to enhance the induction of 5HT fluorescence, and some increase in fluorescence can be achieved by pharmacological manipulation with monoamine oxidase inhibitors to reduce 5HT catabolism — reserpine has been given to reduce amine release, tryptophan has been given to increase availability of precursor in order to increase synthesis, or analogs such as 6-hydroxytryptamine have been administered in order to load the axonal plexuses with fluorescent products.

Another approach to the cellular display of 5HT neurons is the application of immunocytochemistry at the light and electron microscopic levels. In a series of recent studies Pickel and her collaborators (Joh et al., 1975; Pickel et al., 1976; Pickel et al., 1977) raised antibodies against the enzyme tryptophan hydroxylase and then used these for the immunocytochemical localization of serotonin neurons in the raphe nucleus and their axonal processes

in the locus coeruleus. The location of the enzyme was detected by means of the unlabeled primary antibody-peroxidase antiperoxidase method (Sternberger, 1974). These investigators demonstrated that tryptophan hydroxylase is localized (1) to membranes of the endoplasmic reticulum and Golgi apparatus in the cell body, (2) to microtubules in the dendrites and axon and (3) to the surface membranes of small vesicles and large granular vesicles (LGV) in axonal varicosities. They suggested that the association of this enzyme (which catalyzes the initial and probably rate-limiting step in the biosynthesis of 5HT) with the endoplasmic reticulum and the Golgi apparatus represents the site of 5HT synthesis and its association with microtubules indicates the mode of transport of this transmitter.

Another way to display monoamine cells and their terminals is to take advantage of their selective uptake systems for extracellular transmitter and to use autoradiography after the administration of exogenous labeled transmitter. Labeled ^{3}H-5HT can be administered by intraventricular pulse injection (Aghajanian et al., 1966; Fuxe and Ungerstedt, 1968; Bloom et al., 1972), by continuous intraventricular infusion (Chan-Palay, 1975, 1977a), by local instillation (Mouren-Mathieu et al., 1976), or by topical application (Descarries et al., 1975). Such injections are known to produce selective labeling of 5HT neurons and axons because of the high affinity, saturable stereospecific uptake system that 5HT neurons exhibit in biochemical experiments. Morphological data obtained from endogenous fluorescence studies and autoradiography after injections with exogenous ^{3}H-5HT (Fuxe et al., 1968) indicate that comparable structures are demonstrated by the two techniques.

SEROTONIN NEURONS IN THE NUCLEUS PARAGIGANTOCELLULARIS LATERALIS

The 5HT neurons in this nucleus of the medullary reticular formation were recognized as part of a group of yellow fluorescent neurons designated B_3 by Dahlström and Fuxe (1964) in their studies with the Falck-Hillarp method. Electrical stimulation of the PGCL facilitated the firing of raphe pontis and median raphe neurons, presumably by serotoninergic mechanisms (Couch, 1970), a finding which suggests that these two groups of 5HT neurons are linked. Lesions placed in the PGCL followed by an intraventricular pulse injection of ^{3}H-5HT resulted in degenerating synaptic terminals in the raphe pontis, and supported the suggestion that these two 5HT neuron groups interact (Bloom et al., 1972).

Recently the autoradiographic mapping of indoleamine neurons in the brains of the rhesus monkey (Chan-Palay, 1977a) and the rat (Chan-Palay, 1977b) have shown that certain neurons in the caudal PGCL selectively take up ^{3}H-5HT and lie in a neuropil having numerous 5HT axonal terminals.

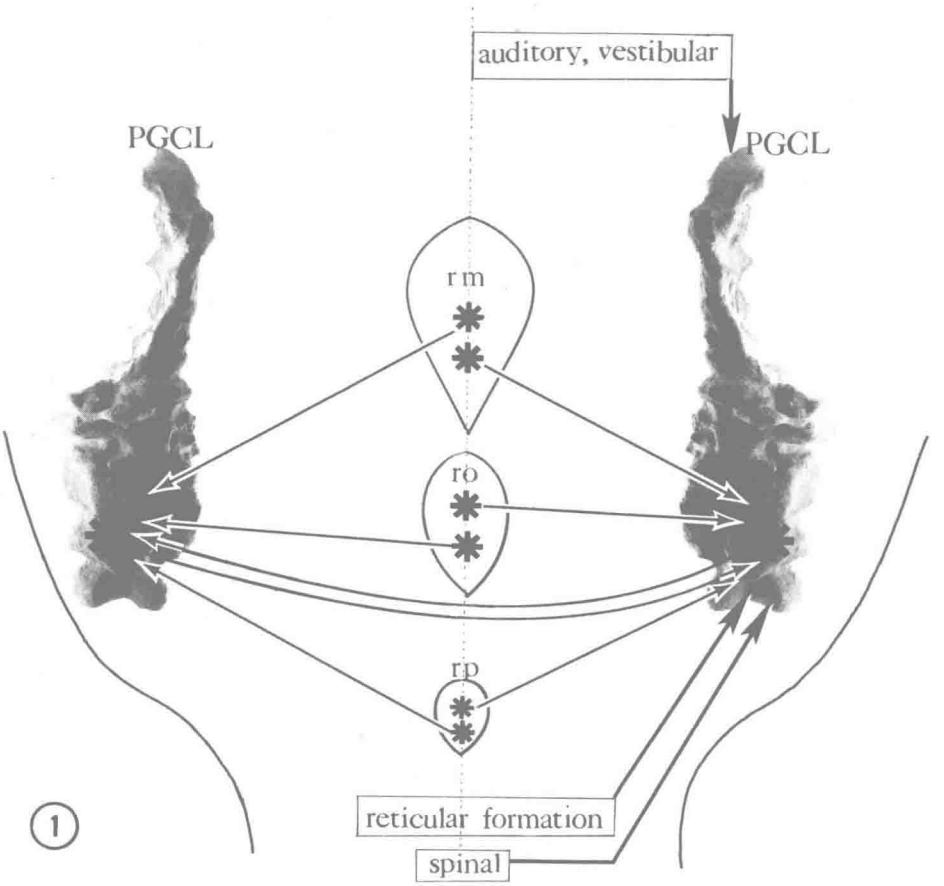


FIGURE 1. A summary of the major inputs to the PGCL nucleus. These include auditory, vestibular, reticular, and spinal inputs, and inputs from the indoleamine neuron-containing nuclei - the raphe magnus (rm), raphe obscurus (ro), raphe pallidus (rp), and the caudal PGCL of the other side of the brain. Modified from Andrezik, Chan-Palay, and Palay, 1977, Fig. 7.

The entire PGCL nucleus in the rat has been carefully defined and reconstructed from serial sections of the brain by Andrezik et al., 1977. A model of the nucleus is reproduced in Figure 1. The

TABLE 2. SOURCES OF INDOLEAMINE AFFERENTS TO PGCL

N. raphe pallidus	2+
N. raphe obscurus	+
N. raphe magnus	3+
N. paragigantocellularis lateralis, contralateral	+

PGCL begins at the level of the rostral magnocellular reticular nucleus at a point approximately at the level of the rostral third of the inferior olivary nucleus. Caudally, where most of the 5HT neurons are found, the PGCL is bounded on its medial aspect by the ventral portion of the nucleus gigantocellularis and laterally by the lateral reticular nucleus and the parvocellular reticular nucleus. The rostral portion of the PGCL abuts dorsally on the nucleus gigantocellularis and the nucleus ambiguus. Laterally it abuts on the motor nucleus of the facial nerve. The caudal, serotonin-containing part of the PGCL measures 1.1 mm at its widest and occupies a large area of the ventral brain stem. It attenuates more rostrally as the model in Figure 1 displays.

In order to facilitate further study of this system of neurons, one needs to know their anatomical connections with other parts of the brain. Retrograde transport of horseradish peroxidase (HRP) injected into the nucleus would allow the mapping of sources of its afferents or inputs, thus providing some indication of such connections. Minute injections of HRP were injected precisely into separate parts of the PGCL, and neurons throughout the brain and spinal cord labeled by HRP after retrograde transport were recorded (Andrezik and Chan-Palay, 1977; Andrezik et al., 1977). These studies demonstrated that the PGCL receives multiple connections from the spinal cord, numerous nuclei of the reticular formation in the pons and medulla, the vestibular nuclei, and several nuclei of the auditory system. In addition, the caudal PGCL in which the 5HT neurons lie, is connected with the PGCL of the other side of the brain, as well as with three other raphe nuclei. These are the raphe obscurus, the raphe magnus and the raphe pallidus, all three of which also contain populations of 5HT neurons (see Dahlström and Fuxe, 1964; Chan-Palay, 1977a, 1977b). Thus, the PGCL is linked to numerous centers in the brain and has possible direct connections with other serotoninergic cell groups in the raphe. These data are summarized in Table 2 and Figure 1. The remainder of this report will be concerned with presentation of fresh evidence from a study of the 5HT neurons of the caudal PGCL with fluorescence microscopy and by autoradiography after intraventricular infusions of ^{3}H-5HT.