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SYNAPTIC NEUROCHEMISTRY OF SENSORY PATHWAYS IN THE
INSECT CENTRAL NERVOUS SYSTEM(U) COLUMBIA UNIV NEW YORK
J G HILDEBRAND 15 MAR 86 ARO-18034.5-LS

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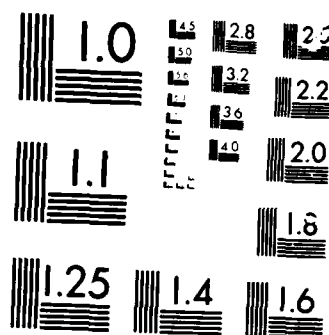
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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) This research project has sought to develop an understanding of the synaptic neurochemistry of important sensory pathways in an experimentally favorable invertebrate central nervous system -- the brain of the moth <i>Manduca sexta</i>. We have concentrated on the olfactory and visual systems because of their importance in this and other animal species (including mankind) and because our knowledge of the functional organization and physiology of these systems is extensive. Our goal has been to add to understanding of the chemical-signalling mechanisms in-		

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20. Abstract, con'd.

involved in information processing in a "model" CNS. In this phase of the research, we have focused effort on identifying important neurotransmitter candidates in the CNS (and the two sensory pathways in particular) and on exploring the "neurochemical anatomy" of the systems. The latter effort has involved the use of histochemical and immunocytochemical methods to probe the cellular localization of several of the important neurotransmitter and neuropeptide candidates in the sensory pathways.

Three principal lines of investigation have been followed during the reporting period. The details of many of our findings have been presented in previous interim technical reports. These lines are:

(1) Chemical probing for neurotransmitter candidates: Using a variety of chromatographic, electrophoretic, and other analytical techniques, we have verified and extended earlier findings about the major transmitter candidates synthesized and retained in the olfactory and visual pathways. Notable among them are: acetylcholine (ACh), gamma-aminobutyric acid (GABA), serotonin (5HT), histamine (HA), and several immunoreactive substances resembling (or identical to) the neuropeptides substance P (SP), corticotrophin-releasing factor (CRF), FMRFamide, adipokinetic hormone (AKH), cholecystokinin (CCK), and leu-enkephalin.

(2) Metabolic studies of GABA: We carried out a thorough study of the uptake of the biosynthetic precursor of GABA -- glutamic acid -- by CNS tissue of Manduca and of the postembryonic development of the biosynthetic and accumulation mechanisms for GABA. This research revealed that the activity of the GABA-synthesizing enzyme glutamate decarboxylase (GAD) increased steadily during postembryonic adult development and was paralleled by an increase in GABA levels. At the time of emergence of the mature adult animal, however, GABA levels increase precipitously over a few hours, and the increase is not explained by an increase in GAD activity. Our findings suggest that several 5-carbon amino acids may serve as metabolic precursors of the glutamate in CNS tissue that is converted by GAD to GABA, and that the sharp increase in GABA at the time of final maturation is due to an abrupt increase in the concentrations of at least some of these precursors in blood and CNS tissue. Studies of glutamate uptake by CNS tissue revealed a dependence on metabolic energy, properties consistent with a carrier-mediated process, and both Na^+ -dependent and independent components.

(3) Neurochemical anatomy, Building upon our neuroanatomical and neurophysiological knowledge of the olfactory pathway in Manduca, as well as on our growing understanding of the types of neurons and their functional organization in that "model" sensory pathway, we have begun to explore the cellular distribution of many of the key neurotransmitter and neuropeptide candidates already mentioned above. Among our main findings are the following: [a] ACh is primarily the transmitter used by the primary sensory fibers entering the CNS from the peripheral sensory organ (the antenna), but some evidence suggests that there may be a few cholinergic central neurons in the primary olfactory center of the brain (the antennal lobe, AL); [b] of the ca. 1000 AL CNS neurons, about 230 exhibit GABA-like immunoreactivity, and most of these cells are local (amacrine) interneurons (LNs) confined to the AL while a few are projection neurons sending axons to the lateral protocerebrum (one of the higher centers for olfactory information processing in the brain); [c] physiological and pharmacological evidence indicates that GABA functions in this system as an inhibitory neurotransmitter acting through a Cl^- mechanism; [d] 5HT is found in one AL neuron; [e] putative neuropeptides revealed in the AL by immunocytochemistry include: SP (>20 neurons), FMRFamide (>1 neuron, possibly a LN but not one containing GABA), CRF (>80 neurons, probably LNs that do not contain GABA), and AKH (>10 LNs).

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I. Introduction and statement of problem studied.

Neurons in animals at all phyletic levels, from the simplest invertebrates to the higher mammals and mankind, communicate with each other and with effector organs such as glands and muscles principally by means of chemical messengers. A central goal of contemporary neurobiology is clarification of the identities, metabolism, cellular distribution, and physiological roles of these chemical messengers. Knowledge about synaptic transmission and extrasynaptic chemical communication in the nervous system promises to lead to many benefits, among which are elucidation and treatment of hitherto intractable neurological and psychiatric disorders, design of new drugs with desirable effects on the nervous system and on innervated end-organs, development of chemical agents to alleviate or protect against the actions of naturally occurring and man-made neurotoxic chemicals, and improved approaches to pest control.

In previous studies in our laboratory and others, it has become increasingly clear that the same substances serve as neurotransmitters, neuromodulators, and neurohormones in both vertebrate and invertebrate nervous systems. Moreover, the cellular mechanisms underlying the formation and functioning of neurons and synapses are remarkably constant at all phyletic levels. Thus there apparently is "one neurochemistry", so that mechanistic insights gained through studies of experimentally favorable invertebrate, vertebrate, and tissue-culture systems can be expected to translate ultimately into principles with wide applicability. Guided by this important doctrine, we study a relatively "simple" invertebrate animal in the hope that it will teach us generally valuable lessons about the organization and functions of all nervous systems. Using a comparative approach, we look for universal mechanisms as well as phyletic differences. Our work not only continues a tradition of using simple systems to facilitate discovery of the universals, but also searches for phyletic peculiarities that might eventually be exploited for design of novel and selective strategies for controlling harmful insects.

This research project has sought to develop an understanding of the synaptic neurochemistry of the olfactory and visual pathways in the central nervous system (CNS) of the experimentally favorable insect Manduca sexta. We have explored the cellular distribution, metabolism, and ontogeny of neurotransmitters and neuropeptides in those pathways. As part of our larger effort to understand the functional organization, physiology, development, and behavioral roles of the chemical senses and vision in Manduca, these neurochemical studies are aided and enhanced by an ever growing body of information about the cells, synapses, and physiology of those sensory pathways. We have pursued three main lines of inquiry in the course of this contract research: (1) chemical probing to determine what putative neurotransmitters are present in the CNS regions of interest to us; (2) studies of the metabolism and development of certain transmitter systems, notably the GABA system; and (3) explorations of the "neurochemical anatomy" of the two sensory pathways (as well as other parts of the CNS) in order to learn about the specific cellular associations of the putative neurotransmitters that are present.

II. Summary of principal findings.

A. Neurotransmitter repertory of the sensory pathways. Through a combination of radiochemical screening, microchemical assays, chromatographic and electrophoretic procedures, and histochemical methods, we have catalogued many of the putative neurotransmitters and neuropeptides present in the olfactory and visual pathways of Manduca. It is clear that the primary olfactory (sensory) neurons, whose cell bodies are in the antenna and whose axons project into the first-order olfactory center in the CNS (the antennal lobe, AL, of the brain), contain acetylcholine (ACh) at high concentrations and are cholinergic in their synaptic interactions with CNS target cells (AL neurons). Pharmacological observations

strongly suggest that the olfactory receptor neurons form nicotinic synapses with AL neurons in specific synaptic sites within the AL neuropil. The ALs produce and accumulate appreciable levels of ACh, gamma-aminobutyric acid (GABA), and histamine (HA), as well as traces of serotonin (5HT) and tyramine, and little or no dopamine, norepinephrine, and octopamine. The visual system presents a somewhat different picture. None of these substances is the likely transmitter candidate in the primary sensory neurons (the photoreceptor cells), and as for photoreceptors in other animals, the primary sensory transmitter in the visual pathway remains unidentified. In screening experiments, the optic lobes of the brain have yielded high levels of ACh and HA, lower levels of GABA, 5HT, and tyramine, and low levels of dopamine and octopamine. No norepinephrine has been detected. The first optic ganglion (the lamina) in the optic lobe appears to use mainly ACh and 5HT (in addition to the unidentified sensory transmitter).

B. Metabolic and developmental studies of a prominent transmitter -- GABA.

Our biochemical and developmental studies during the contract period have focused on some of the processes by which the important inhibitory amino-acid neurotransmitter GABA accumulates in the CNS of Manduca. The production and accumulation of GABA depend on a number of factors, including (1) the supply of glutamate by transport from an exogenous source and by synthesis from precursor amino acids or citric-acid-cycle intermediates, (2) the activity of the biosynthetic and degradative enzymes, and (3) the availability and capacity of mechanisms for storing the GABA intracellularly. To begin to assemble a picture of how these and other factors control neurotransmitter accumulation, we have determined the levels of GABA and other amino acids and of glutamic acid decarboxylase (GAD) activity in the CNS. We quantified a number of substances in the blood that could conceivably serve as carbon sources for GABA synthesis, and we measured the capacity of these substances to contribute to GABA production and to some extent their relative efficiencies in this role. We also studied the uptake of the principal and immediate precursor of GABA synthesis -- glutamate-- into the CNS from exogenous sources. In several aspects of this work, our understanding of the relative importance of different biochemical events in GABA production and accumulation has benefitted from investigation of the developing nervous system of Manduca. In particular, we have paid special attention to the last hours of metamorphic adult development -- the hours immediately before and after emergence of the adult moth. This has allowed us to explore correlations between changes in neurotransmitter levels in the CNS and changes in the biochemical events we believe could be rate-limiting in GABA accumulation.

During postembryonic development of the adult moth, GABA and GAD develop in parallel. Thus, although the activity of the biosynthetic enzyme (GAD) is probably just one factor limiting accumulation of GABA during development, it is the only factor apparent in these studies. After emergence of the adult, however, GABA levels in several regions of the CNS increase dramatically (as much as 50% over a few hours) while extractable GAD activity remains unchanged. This finding suggests that factors in addition to enzyme activity are limiting in GABA production and accumulation. We have found, for example, that other amino acids -- glutamate and proline in particular -- increase in the CNS after emergence in parallel with the increase in GABA levels. We have pursued the notion that these or other 5-carbon amino acids supplied exogenously might participate directly in changes in levels of amino acids in the CNS. The levels of certain such 5-carbon amino acids were quantified in hemolymph before and after adult emergence. While glutamate levels were very low, proline and glutamine were present at concentrations of several millimolar, and perhaps more

importantly, their concentrations increased after adult emergence. That exogenously supplied glutamine and proline, as well as glutamic acid, could serve as carbon sources in GABA synthesis was demonstrated in studies using short-term organ culture in the presence of radioisotopically labeled amino acids. These experiments revealed in addition that exogenous glutamate is largely excluded from some metabolic compartments ("pools") which apparently do not contain GAD activity. Proline and glutamine, however, appear to equilibrate with the CNS pools of those substances in vitro very quickly, and their contributions to GABA synthesis follow this equilibration. It has regrettably not been possible so far to estimate the relative contributions of proline, glutamine, and glutamate to GABA synthesis, owing to the fact that these substances apparently occupy different "metabolic compartments" in the CNS. Nevertheless, the combined results of our studies showed that glutamine and proline are potentially important sources of carbon in GABA synthesis in the CNS and that they are good candidates for a significant role in the rapid increase in CNS GABA levels after emergence.

While the total contribution by glutamate to GABA production remains uncertain for the reasons already outlined and because of the scarcity of glutamate in the blood, the relative exclusion of glutamate from the CNS when supplied exogenously suggested to us that its contribution to GABA production might also be limited by the activity or distribution of carriers for this amino acid in the CNS. We began to pursue this and related issues in a study of the transport of glutamate in the CNS.

We worked out a procedure for studying glutamate transport in in vitro preparations of CNS tissue, optimizing pH and Na^+ concentration and showing linear uptake with short times in vitro. We showed that transport is largely abolished by certain metabolic inhibitors and has a specificity expected of a carrier-mediated process. A Na^+ -dependent component of transport is present in the CNS; apparent kinetic constants have been determined for a low-affinity Na^+ -dependent component of transport and a low-affinity Na^+ -independent component.

Levels of certain amino acids, including glutamate and GABA, increase in CNS structures after emergence of the adult animal but are unchanged in other regions of CNS (such as the abdominal ganglia). The rate of transport of glutamate into the first kind of CNS ganglia increased significantly immediately after emergence of the adult, while the rate of transport in the second kind of CNS ganglia (e.g. abdominal ganglia) was unchanged after emergence.

We presumed that a measured rate of glutamate transport in a CNS ganglion comprises contributions by concentrative uptake and exchange processes. In addition, we believed that the rates we measured would be affected by inter-conversion of amino acids with both retention by the ganglia and release into the medium of those metabolites. We found, in fact, that glutamate is extensively metabolized by CNS tissue and that as little as 20% of the extractable radioactivity in the CNS after a 1-hr incubation with radiolabeled glutamate is in the form of glutamate. A number of radiolabeled amino acids are released into the medium during the course of the incubation, notably proline and glutamine. A comparison of the time-dependent release of these amino acids revealed that newly-synthesized proline makes up a major portion of total proline released early in a one-hour incubation; the release of radiolabeled glutamine is relatively delayed. Thus it appeared to us that the conversion of glutamate to proline, whether for retention or release, is an important metabolic pathway.



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C. Neurochemical anatomy of the olfactory pathway. An important step toward understanding the physiological roles of neurotransmitters and neuropeptides in the CNS is to determine their cellular distribution in parts of the CNS whose functional organization, cell types, and neurophysiology and function are well understood. To pursue the question of roles of such chemical signals, we have studied the cellular distribution of a number of putative transmitters and peptides in the CNS of Manduca and particularly in the olfactory pathway. This work built upon our understanding of the function, neuroanatomy, and cellular neurophysiology of this important sensory pathway in the brain. Among the substances whose cellular distribution we have studied in the moth brain are: the "classical" neurotransmitters acetylcholine (ACh), serotonin (5-hydroxytryptamine, 5HT), gamma-aminobutyric acid (GABA), and dopamine (DA); and the putative neuropeptides proctolin, adipokinetic hormone (AKH), FMRFamide, cholecystokinin (CCK), corticotrophin releasing factor (CRF), leu- and met-enkephalins, substance P (SP), insulin, and glucagon. Most of these likely chemical messengers have been found (or at least immunoreactivities resembling them) in various regions of the CNS. In the olfactory pathway, which we know and have studied most thoroughly, our findings have been as follows. (1) ACh is the transmitter produced, accumulated, and employed by the primary olfactory receptor cells in their synaptic interactions with target neurons in the first-order olfactory center in the brain, the antennal lobe (AL). These primary-sensory synapses are apparently nicotinic in their pharmacology and are all excitatory. (2) ACh is probably also a transmitter of a subset of AL neurons, perhaps one type of projection neurons (PNs). The evidence for this comes mainly from enzyme-cytochemical results indicating that this group of neurons have high levels of intracellular acetylcholinesterase activity -- usually a hallmark of cholinergic neurons. (3) Of the ca. 1000 neurons with somata in the AL, about 230 exhibit GABA-like immunoreactivity, and most of these cells are local (amacrine) interneurons (LNs) confined to the AL. A few of the GABA-immunoreactive cells are PNs that send their axons in a characteristic projection pattern from the AL to the lateral protocerebrum -- an important higher-order center for olfactory information processing in the brain. (4) Physiological and pharmacological evidence strongly supports the idea that GABA functions in the AL as an inhibitory neurotransmitter, acting on GABA receptors that gate Cl^- channels in postsynaptic neurons. Moreover, iontophoretic experiments confirm that cells in the AL respond to GABA with hyperpolarizing (inhibitory) shifts in membrane potential, and these effects are blocked by GABA-antagonistic drugs such as bicuculline and picrotoxinin. (5) 5HT is found in one AL neuron -- an unusual cell that sends its axon to the contralateral AL. The function of this cell is as yet obscure. (6) Putative neuropeptides revealed in the AL by immunocytochemistry so far include: SP (>20 neurons), FMRFamide (>1 neuron, possibly a LN but not one containing GABA), CRF (>80 neurons, probably LNs that do not contain GABA), and AKH (>10 LNs).

IV. Publications resulting wholly or in part from this research.

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16. Kingan TG and Hishinuma A (1986) The transport and metabolism of L-glutamic acid in the abdominal ganglia of the hawkmoth Manduca sexta, in preparation

V. Personnel who have participated in this research project.

[Note: those who have received some salary support from this contract are indicated by *.]

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