Octopamine: Presence in Single Neurons of Aplysia Suggests Neurotransmitter Function

Abstract. *Octopamine has been identified and measured in individual neurons from* Aplysia californica. *Neither dopamine nor norepinephrine was detected in these cells. Thus, in* Aplysia *there may be separate populations of catecholaminergic and monophenolaminergic cells. Octopamine may have functions of its own in the central nervous system of mollusks.*

Physiological and pharmacological observations of molluscan ganglia, including those of *Aplysia californica*, have shown that a number of different neurotransmitter substances may be utilized within these nervous systems (*1*). Several putative neurotransmitters have been identified in single nerve cells from *Aplysia* ganglia, among them serotonin (*2*) and acetylcholine (*3*). Dopamine is also present in the nervous system of *Aplysia* (*4*), but no dopaminergic neurons have been identified as yet.

Octopamine is a biogenic amine formed by β-hydroxylation of tyramine by the enzyme dopamine β-hydroxylase (*5*). It has been found in mammalian nerves (*6*) and ganglia of invertebrates such as octopus and lobster (*7*). In the cockroach nervous system, adenylate cyclase (*8*) and phosphorylase (*9*) can be activated by low octopamine concentrations. These observations suggest that in invertebrate nervous systems octopamine may function as a neurotransmitter.

By the use of a sensitive enzymatic-isotopic micromethod which can easily detect 50 pg of octopamine (*10*), the octopamine content in ganglia and single neurons from *Aplysia californica* has been studied. We report the presence of octopamine in nerve cells that do not contain the catecholamines dopamine and norepinephrine. These results suggest that the octopamine-containing cells are spatially separated from the catecholamine-containing cells and that octopamine may function as a neurotransmitter in *Aplysia*.

Table 1. Octopamine content in the nervous system of *Aplysia*. Octopamine was assayed as described in the text. The results, expressed as picomoles per cluster or area of cells (see text), are means ± standard error of the mean for groups of five different determinations. The molarity of octopamine in a single cell was determined by measuring the diameter of the cell with a calibrated grid and calculating the volume by assuming that the cell was a sphere. For very large, nonspherical cells, longest and shortest diameters were measured; N.D., not detectable.

Ganglia and cells	Octopamine		
	Picomoles per milligram of tissue	Picomoles per cell	Molarity
Abdominal	0.23 ± 0.04		
Bag cells		N.D.	
R1		N.D.	
R2		0.43 ± 0.2	2.5×10^{-6}
R3–13		N.D.	
R14		3.66 ± 2.1	1.5×10^{-4}
R15		N.D.	
R16		N.D.	
L2–6		1.04 ± 0.22	4.6×10^{-5}
L7		1.46 ± 1.0	6.5×10^{-5}
L10		0.11 ± 0.03	1.4×10^{-5}
L11		1.60 ± 1.1	9.1×10^{-6}
L12		N.D.	
L13		0.19 ± 0.03	2.3×10^{-5}
Cerebral	1.85 ± 0.4		
C-1		N.D.	
Cluster A		0.99 ± 0.5	
Cluster B		0.30 ± 0.3	
Cluster C		0.17 ± 0.1	
Buccal	0.85 ± 0.4		
B1		0.11 ± 0.07	3.6×10^{-5}
B2		0.10 ± 0.03	2.2×10^{-5}
B3		0.09 ± 0.03	5.2×10^{-5}
B4		0.10 ± 0.01	1.2×10^{-5}
B5		0.20 ± 0.10	8.9×10^{-5}
B7		1.33 ± 0.27	3.9×10^{-4}
B10		0.25 ± 0.10	2.0×10^{-4}
Pedal	1.05 ± 0.11		
Area A		0.38 ± 0.06	
Area B		0.15 ± 0.15	
Area C		0.46 ± 0.09	
Area D		0.38 ± 0.13	
Pleural	0.20 ± 0.13		
Medial cells		N.D.	

Animals were obtained from Pacific Biomarine Supply Co., Venice, California, and were kept in artificial seawater at 15°C. Ganglia were removed from the animals and frozen on Dry Ice, weighed, and homogenized in 50 volumes of cold 0.2*M* tris(hydroxymethyl)aminomethane hydrochloride (tris-HCl) buffer, *p*H 8.6, containing the monoamine oxidase inhibitor, iproniazid ($1 \times 10^{-3}M$). Single neurons of abdominal ganglia were identified by size, position, and color according to the criteria of Frazier *et al.* (*11*). No attempt was made to identify the cells electrophysiologically. Neurons from the buccal ganglion were identified by the map of Kandel and Gardiner (*12*). The giant cerebral cell, C-1, was identified as described earlier (*2*). Single cells were removed by pinning the stretched ganglia to a layer of Sylgard (Dow Corning) in artificial seawater, carefully slitting the connective tissue capsule, and plucking out the neuron with fine watchmaker's forceps. In order to avoid adherent neuropil, the neuron was held at the region of origin of the axon with both forceps and gently pulled away. Such dissected cells contain a multilayered glial coat, but no synapses (*13*). The total volume contributed by glial cells in such a preparation is very small relative to the nerve cell volume.

Clusters of cell bodies were studied from regions of ganglia where individual neurons could not be consistently identified. In the cerebral ganglia, cluster A consists of a group of about seven large cells near the cerebral-pedal connective. Cluster B is a group of medium-sized cells (about 15) just rostral and medial to cluster A. Cluster C consists of many small cells at the central rostral part of the ganglion, medial to the C-1 cells. In the pedal ganglion, area A consists of a cluster of large cells at the pedal-pedal commissure, area B is a cluster of medium-sized cells at the cerebral-pedal connective, area C is a cluster of small cells near the exit of the posterior-parapodial nerve, and area D is a group of medium-sized cells at the edge of the ganglion opposite area B. The medial pleural cells have been described by Kehoe (*14*). Clus-

ters of cells were removed in the same way as the single cells, but were plucked out several bodies at a time.

After being dissected, the cells were immediately homogenized in 65 μl of cold tris-HCl buffer; two to ten cells were pooled for each sample. After homogenization, ganglia and single cell homogenates were carried through the assay as described elsewhere (*10*). The specificity of the assay was established by identification of the product formed, *N*-methyl octopamine (synephrine), by thin-layer chromatography in three different solvent systems (*10*). More than 95 percent of the radioactivity formed was found to be isographic with synephrine. The catecholamines dopamine and norepinephrine were assayed in ganglia and single cells of *Aplysia californica* by the method of Coyle and Henry (*15*). With this method as little as 25 pg of norepinephrine and 100 pg of dopamine could be measured.

Octopamine was found to be unevenly distributed in the nervous system of *Aplysia californica*. Highest concentrations were found in the buccal ganglion and lowest in the abdominal and pleural ganglia. The cerebral and pedal ganglia showed intermediate concentrations (Table 1). No detectable amounts of octopamine were found in the pleural-abdominal connective, posterior-parapodial nerves, gill, or heart.

Large differences in octopamine concentrations were found in the single neurons examined. Neuron R14 had the highest octopamine concentration (3.66 pmole per cell). Relatively large amounts of octopamine were also found in L2-6, L7, L11, and cell 7 from the buccal ganglion (Table 1). The content of this amine in these cells is of similar magnitude to that of serotonin (*2*) and acetylcholine (*3*) described earlier for single *Aplysia* neurons.

The contents of dopamine and norepinephrine in *Aplysia* ganglion and single cells were also examined. Norepinephrine could not be found in the nervous system of *Aplysia*. Although dopamine was found in *Aplysia* ganglia in concentrations similar to those reported earlier (*4*), this amine was not detected in any of the single cells examined. Although tyrosine hydroxylase and dopamine β-hydroxylase were not directly measured in this study, the absence of dopamine and norepinephrine in neurons that contain high concentrations of octopamine suggests that in *Aplysia* tyrosine hydroxylase and dopamine β-hydroxylase may not coexist in the same cell.

It is likely that octopamine is produced by specific neurons and that this amine has functions of its own in the central nervous system of mollusks and possibly mammals. Recent electrophysiologic studies have shown that at least two receptors exist for octopamine and that these receptors are much more responsive to octopamine than to any other phenylethylamine (*16*). All of these observations indicate that octopamine may function as a neurotransmitter in the nervous system of *Aplysia*.

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Neuronal Analysis of Wave Form in the Time Domain: Midbrain Units in Electric Fish during Social Behavior

Abstract. *A fish of the genus* Eigenmannia *responds differently to a neighboring conspecific fish with a slightly higher frequency of the electric organ discharge than its own than to one with a slightly lower such frequency than its own. When the two frequencies are beating against each other the special wave shape of the electric organ discharge leads to asymmetries of the beat pattern which are distinct for the two cases. Midbrain neurons, called "ΔF decoders," recognize sign and magnitude of the frequency difference on the basis of these patterns, that is, in the time rather than the frequency domain.*

Certain discoveries of new types of feature-detecting afferent neurons are worthy of wider note when they give insight into the mode of analysis of convergent sensory input. Such a neuron has come to light in fish of the genus *Eigenmannia*, not only solving a problem in how the brain distinguishes between two similar stimulus wave forms, each a combination of two frequencies, but pointing to a mechanism that operates in the time domain, that is, looking at the instantaneous form of the beat rather than the constituent frequencies.

Individuals of "wave species" of fish of the family Gymnotidae emit a regular electric organ discharge (EOD) (*1*). The resulting field is a signal source for high frequency–sensitive electroreceptors (*2*) distributed over the body surface. In 1963 Watanabe and Takeda (*3*) studied the species *Eigenmannia virescens*, a gregarious electric fish whose EOD range extends from 200 to 500 hz fundamental frequency. They found that individuals of this species exhibit tonic frequency shifts in response to slightly higher or lower frequencies emitted by electrodes in the water. I have examined the way the brain analyzes the complex combined fields by recording from single neurons in the peripheral and central nervous systems. A type of neuron found in the midbrain will be described here which responds to a small frequency difference (ΔF) between the two fields, down to less than 0.5 hz, and discriminates whether the neighboring fish is higher or lower ($+\Delta F$ or $-\Delta F$) in EOD frequency.

When two *Eigenmannia* of similar frequency are close enough together in space they both shift their frequencies, without trial and error, in the