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OCTOPAMINE: PRESENCE IN SINGLE NEURONS OF *APLYSIA* SUGGESTS NEUROTRANSMITTER FUNCTION

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ABSTRACT

Octopamine has been identified and measured in individual neurons (L7, L11, R14) 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 molluscs.

I. INTRODUCTION

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.[8] Several putative neurotransmitters have been identified in single nerve cells from Aplysia ganglia, among them serotonin[18] and acetylcholine.[11] Dopamine is also present in the nervous system of Aplysia,[1] 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 (DBH).[15] It has been found in mammalian nerves[12] and ganglia of invertebrates such as octopus and lobster.[5,6] In the cockroach nervous system, adenylate cyclase[14] and phosphorylase[16] 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 picograms of octopamine,[13,17] 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 octopamine-containing cells are spatially separated from the catecholamine-containing cells and that octopamine may function as a neurotransmitter in Aplysia.

II. METHODS

Animals were obtained from Pacific Biomarine Supply Company, 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 Tris HCl buffer, pH 8.6, containing 1×10^{-3} M of the monoamine oxidase inhibitor iproniazid. Single neurons of abdominal ganglia were identified by size, position and color according to Frazier et al.[7] Neurons from the buccal ganglion were identified by the map of Kandel and Gardiner.[9] The giant cerebral cell C-1 was identified as described earlier.[18] 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 neuropile, 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.[3] 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.[10] Clusters of cells were removed as were 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; 2 to 10 cells were pooled for each sample. After homogenization, ganglia and single-cell homogenates were carried through the assay as described.[13,17] 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.[13,17] 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.[4] With this method, as little as 25 picograms of norepinephrine and 100 picograms of dopamine could be measured.

III. RESULTS AND DISCUSSION

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 I). No detectable amounts of octopamine were found in the pleural-abdominal connective or posterior-parapodial nerves, the gill and the heart.

Large differences in octopamine concentrations were found in the single neurons examined. R14 had the highest octopamine concentration (3.66 picomoles/cell). Relatively large amounts of octopamine were also found in L2-6, L7, L11 and cell B7 from the buccal ganglion (Table I). The content of this amine in these cells is of similar magnitude to that of serotonin[18] and acetylcholine[11] described earlier for single Aplysia neurons.

Table I. Octopamine Content in the Nervous System of Aplysia*

Ganglia	Cell	Picomoles/mg tissue	Picomoles/cell	Molarity
Abdominal		0.23 ± 0.04		
	Bag cells		not detectable	
	R1		not detectable	
	R2		0.43 ± 0.2	2.5×10^{-6}
	R3-13		not detectable	
	R14		3.66 ± 2.1	1.5×10^{-4}
	R15		not detectable	
	R16		not detectable	
	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		not detectable	
	L13		0.19 ± 0.03	2.3×10^{-5}
Cerebral		1.85 ± 0.4		
	C-1		not detectable	
	Cluster A		0.99 ± 0.5	
	Cluster B		0.30 ± 0.3	
	Cluster C		0.17 ± 0.1	
Buccal		.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		not detectable	

* Octopamine was assayed as described in the text. Results are expressed as means ± SEM for groups of five different determinations. Results are expressed as picomoles per cluster or area of cells (see text). Molarity of octopamine in single cells was determined by measuring the diameter of the cells with a calibrated graticule, and volume was calculated assuming the cell to be a sphere. For very large, nonspherical cells, longest and shortest diameters were measured.

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,[1] this amine was not detected in any of the single cells examined.

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 molluscs 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 any other phenylethylamine.[2] All of these observations indicate that octopamine may function as a neurotransmitter in the nervous system of *Aplysia*.

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