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PUTATIVE NEUROTRANSMITTERS IN APLYSIA: DISTRIBUTION OF GAMMA-AMINOBUTYRIC ACID, ASPARTATE AND GLUTAMATE IN GANGLIA AND SINGLE NEURONS

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PUTATIVE NEUROTRANSMITTERS IN APLYSIA: DISTRIBUTION OF GAMMA-AMINOBUTYRIC ACID, ASPARTATE AND GLUTAMATE IN GANGLIA AND SINGLE NEURONS

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ABSTRACT

The distributions of gamma-aminobutyric acid (GABA), glutamate and aspartate in the nervous system of Aplysia have been measured. GABA was detected in each ganglion examined and in the foot muscle at concentrations ranging from 0.90 ± 0.04 to 1.55 ± 0.17 μmoles/100 mg protein. Aspartate and glutamate were present in greater concentrations in nervous than nonnervous tissues. While ganglia glutamate concentrations were nearly equal, aspartate concentrations varied over twofold among the ganglia. Each identified neuronal cell body examined contained GABA at greater than millimolar concentrations and aspartate at concentrations ranging from 22 ± 3 to 96 ± 11 mM. An apparent correlation was found between the aspartate and GABA content of invertebrate neurons. These results are consistent with a possible neurotransmitter role for amino acids, particularly aspartate and GABA, in Aplysia.

I. INTRODUCTION

The nervous system of Aplysia contains several individual nerve cell bodies which can be reproducibly identified on the basis of their size, position, pigmentation and electrophysiological properties.[8] These neurons range in diameter from 100 μm to nearly 1 mm and can be individually excised for neurochemical determinations or penetrated with microelectrodes for electrophysiological investigations. Information gained from these types of studies has indicated marked heterogeneity among this population of neurons. For example, of the 30 to 40 identifiable neurons, only four, L10, L11, R2 and the left pleural giant cell (LPGC), have been found to contain choline acetyl transferase (acetyl-CoA:choline-O-acetyl transferase, EC 2.3.1.6).[12] Subsequent studies have shown that these four neurons are uniquely capable of synthesizing acetylcholine[6] and do, in fact, contain greater amounts of this neurotransmitter than do other identified neurons.[25] Other investigations have shown that two other neurons, the paired giant cerebral cells (C-1), are capable of synthesizing 5-hydroxytryptamine (serotonin)[6] and contain this putative neurotransmitter in concentrations significantly greater than found in other neurons.[33]

The unique occurrence in a particular neuron of either acetylcholine or serotonin and the enzymes required for their biosynthesis has traditionally been used to designate that neuron as either cholinergic or serotonergic, respectively. However, glutamate, which may be a neurotransmitter in invertebrates,[16] has been shown to be evenly distributed among Aplysia neurons.[3] Moreover, recent findings in our laboratory indicate that some of the identified Aplysia neuronal cell bodies contain

more than one putative neurotransmitter.[4,30] A more complete description of the distribution of putative neurotransmitters in Aplysia identified neurons may offer a better understanding of (1) the function of these agents in individual neurons, and (2) the interrelationships existing between heterogeneous neurons in the same nervous system. In this report we present data describing the distribution of three amino acid neurotransmitter candidates, gamma-aminobutyric acid (GABA), glutamate and aspartate, in ganglia and individual identified nerve cell bodies in Aplysia.

II. METHODS

Animals. Aplysia californica and Aplysia dactylomela (200-500 g) were obtained from commercial suppliers and were housed in tanks of artificial seawater at 17°C until the time of the experiment. The animals were pinned to dissecting trays and the ganglia removed. Individual ganglia were pinned to a Sylgard layer in a Lucite chamber under a dissecting microscope and the connective tissue sheath slit with a razor blade.

Regional distribution. For determination of regional distributions, whole ganglia were dissected free of the ganglia sac connective tissue and homogenized in 50 µl of ice-cold 80 percent (v/v) ethanol to precipitate proteins. Samples (1-5 mg) of foot muscle and ganglia sac connective tissue were treated similarly. After centrifugation at 5000 x g for 20 min, the pellets were washed twice with additional aliquots of 80 percent ethanol. The pellets were then suspended in 0.1 N NaOH for total protein determination by the method of Lowry et al.[22] The combined supernatant fractions were evaporated to dryness at 60°C under reduced pressure and

were resuspended in H_2O. Portions of this aqueous extract were assayed for GABA, glutamate and aspartate as described below.

Single neuron distribution. Individual neuronal cell bodies were identified on the basis of their size, position and pigmentation according to reported descriptions [8,9,14,33] and were dissected as previously described.[30] The neuronal cell bodies dissected in this fashion are covered with a glial investment but are presumably free of synaptic contacts, since these are located primarily in the neuropile.[5] Sometimes small neurons adhere tightly to the larger cell bodies. These were for the most part carefully dissected free. It is unlikely that neuronal and glial contamination of single cell samples amounted to more than 5 percent of the sample volume. Positive identification of some of the neurons (L1, L7-L9, B4-B9) requires electrophysiological characterization, which was not performed in these experiments. Although we did not remove cells unless their gross characteristics were as expected from published descriptions, it is possible that some errors in identification were made. The minimum and maximum diameters of each cell, and thus its volume, were determined at the time of dissection (Table I).

Each cell was homogenized in 20-50 µl of 0.1 N HCl. Portions of the homogenate were stored at -20°C and lyophilized just before the time of assay. Most cells contained sufficient aspartate to allow both duplicate and blank assays on each cell. In some experiments, GABA assays were done on the lyophilization residue of cells which had been frozen intact in 10 µl of 0.1 N HCl or homogenates of cells pooled from two or three animals.

Table I. Volume of Identified Aplysia Neuronal Cell Bodies

Neuron*	Volume (10^{-9} l)†	
L1	13 ± 5	(5)
L2-L6‡	47 ± 4	(22)
L7	24 ± 2	(11)
L11	29 ± 2	(11)
L8-L9‡	6 ± 1	(3)
R2	84 ± 9	(15)
R3-R13‡	17 ± 4	(7)
R14	28 ± 4	(6)
R15	18 ± 2	(7)
C-1	14 ± 4	(6)
LPGC	97 ± 11	(13)
B1	7 ± 1	(12)
B2	8 ± 1	(17)
B3	5 ± 1	(8)
B4-B9‡	2.8 ± 0.1	(9)

* Individual neuronal cell bodies were identified and are designated by reported descriptions (see Methods)

† Volumes were calculated from measurements of the minimum and maximum diameters of each neuron made at the time of dissection. Values are the mean ± S.E.M. for the number of cells indicated in parentheses.

‡ Cell bodies in these groups were not individually distinguished

Biochemical assays. GABA assays and blank assays were done by the enzymatic-fluorometric procedure described by Kravitz et al.[19] The GABASE system enzymes (4-aminobutyrate:2-oxoglutarate aminotransferase, EC 2.6.1.19 and succinate-semialdehyde:NAD (P) oxidoreductase, E.C.1.2.1.16) were extracted from suitably grown Pseudomonas fluorescens (A.T.C.C. 13430; obtained as a frozen cell paste from International Mining and Chemical Corporation, Skokie, Illinois) as

described by Jakoby.[15] Aspartate and glutamate assays and blank assays were conducted by the enzymatic-fluorometric procedures of Graham and Aprison[13] except that the glycine-hydrazine buffer used in the glutamate assay was replaced by 200 mM Tris pH 8.2 containing 0.42 percent (w/v) hydrazine hydrate. Other enzymes and pyridine nucleotides were obtained from Sigma Chemical Company, St. Louis, Missouri. All fluorescence measurements were made with an Aminco Bowman spectrophotofluorometer at excitation and emission wavelengths of 365 and 460 nm, respectively.

Aplysia species differences. In each of the following studies, no apparent differences were observed between data obtained from the two Aplysia species used. Therefore, each study includes data obtained from both species. The majority of the single cell data was obtained from Aplysia californica because this species afforded more certain identification of several of the individual neurons.

III. RESULTS

Regional distributions. Glutamate was distributed evenly among Aplysia ganglia (12.0 to 16.0 μmoles/100 mg protein) but was only about one-fourth as concentrated in the ganglia sac connective tissue and foot muscle (Table II). Aspartate, like glutamate, was more highly concentrated in nervous than nonnervous tissues. However, there was a wider range of aspartate values among the different ganglia, with the highest concentration (buccal) being over twice that of the lowest (cerebral). GABA was present in each of the ganglia and in the foot muscle at concentrations ranging from 0.90 to 1.55 μmoles/100 mg protein. There was little or no GABA in the ganglia sac

Table II. Distribution of GABA, Glutamate and Aspartate in Aplysia*

	Glutamate	GABA	Aspartate
Ganglia			
Abdominal	12.4 ± 2.3	1.19 ± 0.43	19.5 ± 1.2
Buccal	16.0 ± 2.8	1.55 ± 0.17	29.1 ± 5.6
Cerebral	14.5 + 2.0	1.17 ± 0.08	13.7 ± 1.8
Pedal	12.0 ± 1.0	0.90 ± 0.04	19.1 ± 2.2
Pleural	15.8 ± 2.5	1.18 ± 0.30	21.4 ± 1.7
Ganglia sacs	4.4 ± 0.2	< 0.5	3.7 ± 0.4
Foot muselc	3.2 ± 0.3	0.99 ± 0.17	5.5 ± 0.5

* Values are expressed as μmole/100 mg protein, and are the mean ± S.E.M. for three to seven determinations. For experimental details see Methods.

conneetive tissue. In eight samples analyzed two contained no GABA (< 0.25 μmole/100 mg protein) while the othcrs ranged from 0.3 to 0.7 μmolc/100 mg protein.

GABA in single identified neuron cell bodies. Since the minimum sensitivity of the fluorometric GABA assay was about 25 pmoles, a neuron with a volume of 25 nl would eontain measurable GABA only if its endogenous eoneentration were greater than 1 mM. To achieve an approximatc minimum sensitivity level of 1 mM, those eells, the average volume of whieh was in exeess of 25 nl, were assayed singly. Smaller neurons werc often pooled for eaeh assay. Tissue blank assays conducted on scveral of the larger neurons yieldcd fluorescenee values similar to reagent blanks.

The results of thc GABA assays of identified Aplysia neuron ecll bodies are shown in Table III. Each eell body assayed eontained GABA in greater than millimolar eoncentrations. With the exeeption of L1, the neurons of the abdominal ganglion had the lowest GABA eoneentrations, ranging from 1.3 to 2.7 mM. The buecal ganglion, whieh had the highest whole-ganglion GABA eoneentration, also eontained individual neuron eell bodies having GABA coneentrations grcater than those in other

Table III. GABA Content of Identified Aplysia Neuronal Cell Bodies

Neuron	GABA	
	pmole/cell	mM
L1	99, 54 (3)	6.4
L2-L6	95, 57, 57, 28, 71 (2), 42 (6)	1.3 ± 0.2
L7	37, 60, 41, 34 (2)	2.0 ± 0.2
L11	112, 37, 33, 53 (2)	1.4 ± 0.2
R2	259, 197, 255, 148, 107	1.8 ± 0.3
R3-R13	77, 26 (3), 27 (5), 25 (6)	2.7 ± 0.8
R14	134, 53 (2)	2.7
R15	63, 48, 26, 37, 87, 48 (2)	2.2 ± 0.4
C-1	55, 84, 78, 26, 48 (2), 78	4.1 ± 1.3
LPGC	336, 295, 82, 134, 152, 49	1.6 ± 0.4
B1	49, 55, 29 (3)	5.7 ± 0.7
B2	<25, 27 (5), 37, 26	3.3 ± 0.4
B3	<25, 24 (6)	5.2
B4-B9	18 (7), 19 (4), 16 (5), 10 (19)	8.0 ± 1.7

* Each individual determination (pmole/cell) is the result of a single assay of a separate cell except where indicated by the number (in parentheses) of cells pooled for a single assay. Concentrations (mM) are the mean ± S.E.M. of the concentrations determined for each cell from its respective volume.

ganglia. The group of small buccal cells B4-B9 contained the largest GABA concentrations encountered (8.0 ± 1.7 mM).

Aspartate in single identified neuron cell bodies. Each nerve cell body assayed contained in excess of 300 pmoles of aspartate (Table IV), and concentrations varied considerably among the neurons. Seven of the fifteen neurons studied contained 20 to 35 mM aspartate, while five contained greater than 70 mM. Aspartate concentrations in the buccal neurons were consistently higher than in those of other ganglia. However, both the highest and lowest concentrations were found in the abdominal ganglion.

Neurons L8-L9 (96 ± 11 mM) contained over four times the concentration of aspartate found in R3-R13 (22 ± 3 mM).

Table IV. Aspartate Content of Identified Aplysia Neuronal Cell Bodies

Neuron	Number assayed	Aspartate	
		pmole/cell	mM
L1	3	581 ± 193	71 ± 24
L2-L6	14	1152 ± 126	28 ± 3
L7	5	771 ± 135	34 ± 7
L8-L9	3	504 ± 59	96 ± 11
L11	8	1237 ± 153	44 ± 4
R2	10	1746 ± 319	25 ± 4
R3-R13	4	470 ± 59	22 ± 3
R14	4	586 ± 56	27 ± 3
R15	2	833	47
C-1	9	365 ± 47	34 ± 5
LPGC	7	2597 ± 458	34 ± 5
B1	7	307 ± 35	49 ± 6
B2	11	517 ± 73	85 ± 9
B3	5	371 ± 60	77 ± 16
B4-B9	5	319 ± 101	90 ± 22

* Total aspartate contents (pmole/cell) are the mean ± S.E.M. of assays of portions of single cell homogenates (see Methods). Concentrations (mM) are the mean ± S.E.M. of the concentrations determined for each cell from its respective volume.

IV. DISCUSSION

GABA has been reported to be present in molluscan nervous systems and muscle by Osborne et al.[26] in Helix and by Abramson et al.[1] in the abdominal ganglion of Aplysia. In Crustacea in which the neurotransmitter roles of GABA and glutamate

have been best characterized,[16,27] individual ganglia have been shown to contain GABA at concentrations of 1 to 3 μmoles/g, glutamate at 10 to 20 μmoles/g, and aspartate at 15 to 50 μmoles/g.[2,7] Data reported here indicate that these three amino acids are similarly distributed in the nervous system of Aplysia.

The widespread occurrence of GABA at greater than millimolar concentrations in individual neuronal cell bodies is not easily interpreted in light of previous investigations. Kravitz and Potter[20] found that the GABA concentration in inhibitory axons of the lobster was in excess of 100 mM, and the cell bodies of the same neurons contained 10 to 20 mM.[29] Individual lobster excitatory neuronal cell bodies and axons contained 1 mM GABA or less. Based on these findings one would expect a clear difference in GABA concentration between neurons releasing GABA as a neurotransmitter and other neurons which do not. This interpretation may be warranted for the Aplysia buccal ganglion cell bodies B4-B9, which contained 8 mM GABA and of which some are known to provide inhibitory synaptic input to other buccal neurons.[9] Moreover, L11, R2 and the left pleural giant cell (LPGC), which are thought to be cholinergic, were among the neurons with the lowest GABA content. However, the gradation of concentrations found among the other neurons precludes such direct and specific designations.

Aspartate concentrations also varied over a relatively wide range among the identified neurons. Individual neuronal cell body aspartate concentrations have not been previously determined for other species, but measurements of whole ganglion and axonal aspartate concentrations in the lobster and crab indicate similar wide ranges.[2,7,24] Aprison et al.[2] recently observed that those lobster ganglia with

high aspartate contents also contained GABA at higher concentrations than did other ganglia. In Figure 1, we have plotted our GABA and aspartate data together with those of Aprison et al. to demonstrate this relationship. Analysis of these combined data yields a significant linear correlation coefficient ($r = 0.78$, $n = 25$, $p < 0.001$) between the aspartate and GABA contents of these invertebrate ganglia and neuronal cell bodies.

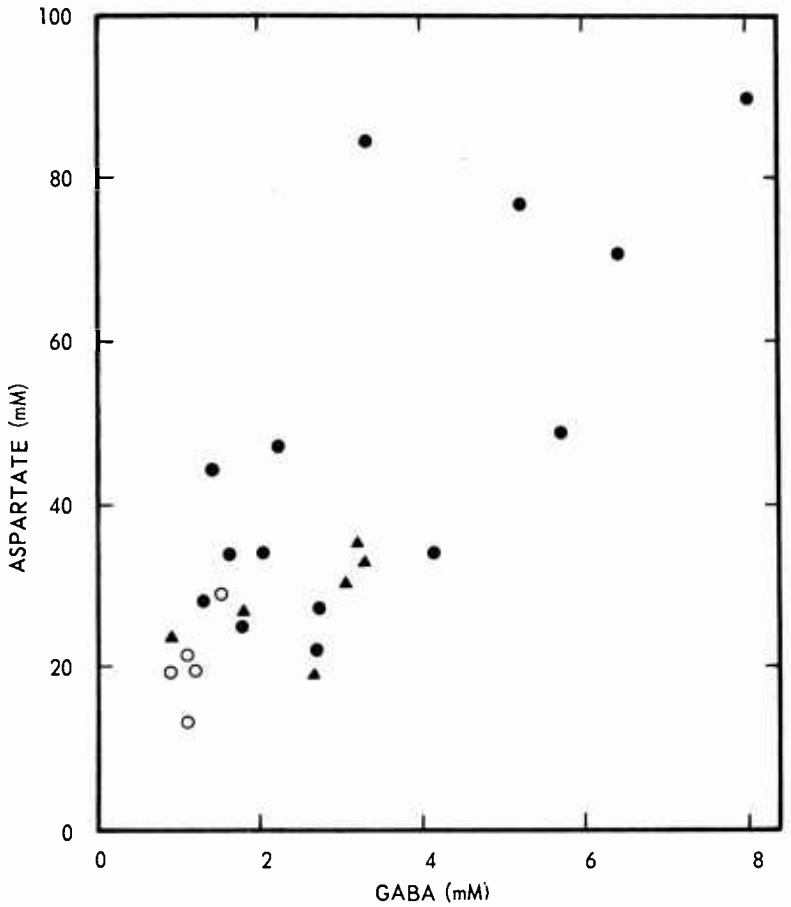

Figure 1. Aspartate versus GABA content of lobster and Aplysia neurons and ganglia. Data from our regional distribution study of Aplysia ganglia (o) and neurons (•) are plotted together with data from the regional distribution study of lobster ganglia (▲) reported by Aprison et al.[2] The units mM, mmole/kg and μmole/100 mg protein have been assumed to be equivalent.

This correlation between the GABA and aspartate contents of nerve ganglia and neuronal cell bodies does not seem to apply to regions of axons distant from cell bodies. Marks et al.[23] found that the GABA concentration of more distal sections of lobster nerve fibers was only about 25 percent of that found in proximal sections, while the aspartate content remained relatively constant. Additionally, McBride et al.[24] found that while four giant axons of lobster contained no GABA, they did contain in excess of 50 mM aspartate. There does not appear to be any correlation between GABA (or aspartate) concentrations and the concentrations of the other amino acids in lobster ganglia,[2] or with glutamate concentrations either in whole Aplysia ganglia (our data) or identified neurons.[3] The lack of correlation between GABA and glutamic acid is particularly striking, since GABA is synthesized from glutamic acid, whereas aspartate is only indirectly connected to this pathway through the citric acid cycle.

Recent investigations have shown that a single neuronal cell body of Aplysia may contain serotonin, histamine, octopamine, and glutamate as well as acetylcholine.[4] Although presence of a putative neurotransmitter by no means documents release of that substance by nervous excitation, these observations suggest that multiple transmitters may be released from the same terminal and that they may interact at both presynaptic and postsynaptic sites. GABA release from stimulated inhibitory lobster axons was demonstrated by Otsuka et al.[28] Release of glutamate has been demonstrated from stimulated nerve muscle preparations of several invertebrate species[17,32] while lesser amounts of aspartate, glycine and alanine were also released. The proportionate distribution of GABA and aspartate reported here may indicate that these

two amino acids are simultaneously released from certain Aplysia nerve terminals and interact in a physiologically important manner at either presynaptic or postsynaptic membranes.

Shank and Freeman[31] have suggested a similar interaction between aspartate and glutamate at the lobster neuromuscular junction. In preliminary electrophysiological investigations, they found that while aspartate is much less effective than glutamate at depolarizing the muscle fiber, it potentiates the action of glutamate. They proposed that both compounds are released from the nerve terminal and act in a postsynaptic complex which includes specific receptors for both aspartate and glutamate. They suggest that the primary function of the aspartate receptor is to increase the affinity between glutamate and its receptor.

Gerschenfeld and Tauc,[11] found that certain Aplysia neurons were hyperpolarized by externally applied GABA, while others were depolarized. Similar results obtained in the land snail by Gerschenfeld and Lasansky[10] suggest the existence of specific receptors in neuronal cell body membranes which mediate either hyperpolarizing or depolarizing responses to externally applied GABA, and other receptors to glutamate and aspartate. Preliminary electrophysiological investigations in our laboratory have indicated that specific receptors may be found in Aplysia ganglia mediating a depolarizing Na^+ conductance increase response to GABA and two types of hyperpolarizing responses due to conductance increases to Cl^- and K^+, respectively (Yarowsky and Carpenter, unpublished results).

It is possible that the primary function of the high concentrations of aspartate and glutamate in invertebrate nerve is to achieve charge balance and maintain osmotic equilibrium. From experiments measuring the free amino acids of crab and lobster leg nerves as well as Sepia giant axons, Lewis[21] concluded that aspartate and glutamate were free in axoplasm and in high enough concentrations in all three tissues to balance two-thirds of the internal K^+. In all these tissues, aspartate was two to four times as concentrated as glutamic acid and was the primary organic anion. In squid giant axon, the primary anion is isethionate at 220 μeq/g, but aspartate is the second most concentrated at 65 μeq/g.[18] Isethionate has not been described in other invertebrate nervous systems. Lewis[21] and Evans[7] note the presence of large amounts of taurine in invertebrate nerves, and this substance may contribute significantly to osmotic balance. Variation in aspartate content from neuron to neuron, as demonstrated in our experiments, implies the existence of other inorganic or organic ionic differences between neurons, since both osmotic and ionic balance must be maintained. The discrepancy between the Aplysia neurons with the highest and lowest aspartate concentrations is 74 mM. This dramatic difference between neurons may have some functional significance related to neurotransmitter function, although it is not yet possible to implicate aspartate over whatever substances make up the osmotic and ionic balance in neurons with low aspartate content. We plan electrophysiological studies with aspartate to attempt to answer this question.

It is likely that amino acids serve multiple functions in the invertebrate nervous system. GABA, aspartate and glutamate are present in all Aplysia neurons examined, but GABA and aspartate are unequally distributed. It is those neurons with the higher

concentrations which are the most likely to release GABA and aspartate. If these substances are neurotransmitters then the crucial sites are the presynaptic terminals, which must package and release them, and the postsynaptic membranes, which must contain the receptors. The analysis of putative neurotransmitter content in neuronal cell bodies cannot by itself prove a transmitter function, but the results of this study are not inconsistent with a neurotransmitter role for amino acids, particularly GABA and aspartate, in Aplysia.

REFERENCES

1. Abramson, F. P., McCaman, M. W. and McCaman, R. E. Femtomole level of analysis of biogenic amines and amino acids using functional group mass spectrometry. Anal. Biochem. 57:482-499, 1974.

2. Aprison, M. H., McBride, W. J. and Freeman, A. R. The distribution of several amino acids in specific ganglia and nerve bundles of the lobster. J. Neurochem. 21:87-95, 1973.

3. Borys, H. K., Weinreich, D. and McCaman, R. E. Determination of glutamate and glutamine in individual neurons of Aplysia californica. J. Neurochem. 21: 1349-1351, 1973.

4. Brownstein, M. J., Saavedra, J. M., Axelrod, J., Zeman, G. H. and Carpenter, D. O. Coexistence of several putative neurotransmitters in single identified neurons of Aplysia. Proc. Natl. Acad. Sci. USA 71:4662-4665, 1974.

5. Coggeshall, R. E. A light and electron microscope study of the abdominal ganglion of Aplysia californica. J. Neurophysiol. 30:1263-1287, 1967.

6. Eisenstadt, M., Goldman, J. E., Kandel, E. R., Koike, H., Koester, J. and Schwartz, J. H. Intrasomatic injection of radioactive precursors for studying transmitter synthesis in identified neurons of Aplysia californica. Proc. Natl. Acad. Sci. USA 70:3371-3375, 1973.

7. Evans, P. D. Amino acid distribution in the nervous system of the crab, Carcinus maenas. J. Neurochem. 21:11-17, 1973.

8. Frazier, W. T., Kandel, E. R., Kupfermann, I., Waziri, R. and Coggeshall, R. E. Morphological and functional properties of identified neurons in the abdominal ganglion of Aplysia californica. J. Neurophysiol. 30:1288-1351, 1967.

9. Gardner, D. Bilateral symmetry and interneuronal organization in the buccal ganglia of Aplysia. Science 173:550-553, 1971.

10. Gerschenfeld, H. M. and Lasansky, A. Action of glutamic acid and other naturally occurring amino-acids on snail central neurons. Int. J. Neuropharmacol. 3:301-314, 1964.

11. Gerschenfeld, H. and Tauc, L. Pharmacological specificities of neurones in an elementary central nervous system. Nature 189:924-925, 1961.

12. Giller, E., Jr. and Schwartz, J. H. Choline acetyltransferase in identified neurons of abdominal ganglion of Aplysia californica. J. Neurophysiol. 34:93-107, 1971.

13. Graham, L. T., Jr. and Aprison, M. H. Fluorometric determination of aspartate, glutamate, and γ-aminobutyrate in nerve tissue using enzymic methods. Anal. Biochem. 15:487-497, 1966.

14. Hughes, G. M. and Tauc, L. An electrophysiological study of the anatomical relations of two giant nerve cells in Aplysia depilans. J. Exp. Biol. 40:469-486, 1963.

15. Jakoby, W. B. Enzymes of γ-aminobutyrate metabolism (bacterial). In: Methods in Enzymology, Colowick, S. P. and Kaplan, N. O., editors, Vol. V, pp. 765-778. New York and London, Academic Press, 1962.

16. Johnson, J. L. Glutamic acid as a synaptic transmitter in the nervous system. A review. Brain Res. 37:1-19, 1972.

17. Kerkut, G. A., Leake, L. D., Shapira, A., Cowan, S. and Walker, R. J. The presence of glutamate in nerve-muscle perfusates of Helix, Carcinus and Periplaneta. Comp. Biochem. Physiol. 15:485-502, 1965.

18. Koechlin, B. A. On the chemical composition of the axoplasm of squid giant nerve fibers with particular reference to its ion pattern. J. Biophys. Biochem. Cytol. 1:511-529, 1955.

19. Kravitz, E. A., Kuffler, S. W. and Potter, D. D. Gamma-aminobutyric acid and other blocking compounds in Crustacea. III. Their relative concentrations in separated motor and inhibitory axons. J. Neurophysiol. 26:739-751, 1963.

20. Kravitz, E. A. and Potter, D. D. A further study of the distribution of γ-aminobutyric acid between excitatory and inhibitory axons of the lobster. J. Neurochem. 12:323-328, 1965.

21. Lewis, P. R. The free amino-acids of invertebrate nerve. Biochem. J. 52:330-338, 1952.

22. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265-275, 1951.

23. Marks, N., Datta, R. K. and Lajtha, A. Distribution of amino acids and of exo- and endopeptidases along vertebrate and invertebrate nerves. J. Neurochem. 17:53-63, 1970.

24. McBride, W. J., Freeman, A. R., Graham, L. T., Jr. and Aprison, M. H. The content of several amino acids in the external cell sheath and four giant axons of a nerve bundle from the CNS of the lobster. Brain Res. 59:440-444, 1973.

25. McCaman, R. E., Weinreich, D. and Borys, H. Endogenous levels of acetylcholine and choline in individual neurons of Aplysia. J. Neurochem. 21:473-476, 1973.

26. Osborne, N. N., Briel, G. and Neuhoff, V. Distribution of GABA and other amino acids in different tissues of the gastropod mollusc Helix pomatia, including in vitro experiments with ^{14}C glucose and ^{14}C glutamic acid. Int. J. Neurosci. 1:265-272, 1971.

27. Otsuka, M. γ-Aminobutyric acid in the nervous system. In: The Structure and Function of Nervous Tissue, Bourne, G. H., editor, Vol. IV, pp. 249-289. New York and London, Academic Press, 1972.

28. Otsuka, M., Iversen, L. L., Hall, Z. W. and Kravitz, E. A. Release of gamma-aminobutyric acid from inhibitory nerves of lobster. Proc. Natl. Acad. Sci. USA 56:1110-1115, 1966.

29. Otsuka, M., Kravitz, E. A. and Potter, D. D. Physiological and chemical architecture of a lobster ganglion with particular reference to gamma-aminobutyrate and glutamate. J. Neurophysiol. 30:725-752, 1967.

30. Saavedra, J. M., Brownstein, M. J., Carpenter, D. O. and Axelrod, J. Octopamine: presence in single neurons of Aplysia suggests neurotransmitter function. Science 185:364-365, 1974.

31. Shank, R. P. and Freeman, A. R. Interaction of glutamate and aspartate with receptors on the neuromuscular excitatory membrane in the walking limb of the lobster. Fed. Proc. 33:449 (Abstract 1349), 1974.

32. Usherwood, P. N. R., Machili, P. and Leaf, G. L-glutamate at insect excitatory nerve-muscle synapses. Nature 219:1169-1172, 1968.

33. Weinreich, D., McCaman, M. W., McCaman, R. E. and Vaughn, J. E. Chemical, enzymatic and ultrastructural characterization of 5-hydroxytryptamine-containing neurons from the ganglia of Aplysia californica and Tritonia diomedia. J. Neurochem. 20:969-976, 1973.

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11. SUPPLEMENTARY NOTES

12. SPONSORING MILITARY ACTIVITY
Director
Defense Nuclear Agency
Washington, D. C. 20305

13. ABSTRACT

The distributions of gamma-aminobutyric acid (GABA), glutamate and aspartate in the nervous system of Aplysia have been measured. GABA was detected in each ganglion examined and in the foot muscle at concentrations ranging from 0.90 ± 0.04 to 1.55 ± 0.17 μmoles/100 mg protein. Aspartate and glutamate were present in greater concentrations in nervous than nonnervous tissues. While ganglia glutamate concentrations were nearly equal, aspartate concentrations varied over twofold among the ganglia. Each identified neuronal cell body examined contained GABA at greater than millimolar concentrations and aspartate at concentrations ranging from 22 ± 3 to 96 ± 11 mM. An apparent correlation was found between the aspartate and GABA content of invertebrate neurons. These results are consistent with a possible neurotransmitter role for amino acids, particularly aspartate and GABA, in Aplysia.

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