Cyclic Adenosine Monophosphate in the Nervous System of *Aplysia californica*

I. *Increased synthesis in response to synaptic stimulation*

HOWARD CEDAR, ERIC R. KANDEL, and JAMES H. SCHWARTZ

From the Departments of Pathology, Physiology, Psychiatry, and Microbiology, New York University Medical Center and the Public Health Research Institute of the City of New York, New York 10016

ABSTRACT In the isolated abdominal ganglion of *Aplysia*, previously incubated in adenine-^{3}H, the amount of ^{3}H-labeled adenosine-3′,5′ monophosphate (cAMP) doubled after electrical stimulation of nerves at a physiological rate (1/sec). No change was detected after 4 min of stimulation. An increase in cAMP was first seen after 15 min; lengthening the period of stimulation to 1 hr did not increase the extent of the effect. ATP contained 50% of the total radioactivity taken up from adenine-^{3}H, cAMP about 0.1%. During stimulation both the total amount and the specific radioactivity of adenosine triphosphate (ATP) did not change. Thus, the increased amount of radioactivity found in cAMP after stimulation represented an increase in its rate of synthesis. During stimulation formation of cAMP-^{3}H was not altered in nerves or in the cell body of an identified neuron (R2). In addition, no changes were detected in the total amounts of cAMP in the ganglion and in the cell body of R2. It seems likely that the increase was initiated by synaptic activity rather than by action potentials. It was blocked by elevating the concentration of Mg, which also blocks synaptic activity without impairing conduction of impulses. Moreover, impulse activity induced by ouabain and glutamate did not result in increased formation of cAMP.

INTRODUCTION

In addition to generating synaptic potentials, transmission at chemical junctions may also produce metabolic changes in the postsynaptic neuron (see, for example, Larabee and Leicht, 1965; Berry, 1969; Peterson and Kernell, 1970; Kernell and Peterson, 1970). These biochemical concomitants of synaptic transmission may be important for maintenance of synaptic activity or for plastic interactions between neurons. Adenosine 3′,5′ monophosphate

(cAMP) may mediate some of the biochemical changes which follow synaptic transmission. Neuronal activity can, in fact, lead to an increase in cAMP in nervous tissues (see Greengard and Costa, 1970). Thus, brain slices that are exposed to depolarizing agents or to biogenic amines, or that are electrically stimulated with direct current, show large increases in cAMP (Kakiuchi and Rall, 1968 *a*, *b*; Kakiuchi, Rall, and McIlwain, 1969; Shimizu, Creveling, and Daly, 1970 *a*, *b*). The physiological meaning of these effects is obscure, however, because the architectural complexity of brain slices and the artificial nature of the stimulation make interpretation difficult. It would be important to know whether cAMP increased in all types of cells (neurons and glia) or only in some; if neuronal, whether the increase resulted from impulse activity or from synaptic stimulation; and, if it was the result of synaptic stimulation, whether the increase was a general response or one which could be evoked only by certain specific transmitter substances. An approach to these problems has been made by McAfee, Schorderet, and Greengard (1971) who found that preganglionic stimulation of the superior cervical ganglion of the rabbit for brief periods produced a several-fold increase in total content of cAMP. They suggested that cAMP was increased in response to the synaptic release of catecholamine from internuncial neurons in the ganglion.

Because of the wealth of information available about different types of synaptic activity mediated by a variety of transmitter substances in the abdominal ganglion of the marine gastropod mollusc *Aplysia* (see Gerschenfeld, 1966; Tauc, 1967), we have used this preparation to examine the biochemical consequences of synaptic activity. We have found that electrical stimulation of peripheral nerves and connectives of the ganglion leads to increased synthesis of cAMP, and present some evidence to suggest that this results from synaptic stimulation. In the accompanying paper, Cedar and Schwartz show that of several known putative transmitter substances, only serotonin and dopamine stimulate the formation of cAMP.

MATERIALS AND METHODS

Aplysia californica were supplied by Dr. R. C. Fay of the Pacific Bio-Marine Supply Co., Venice, Calif. When fed with seaweed, animals could be kept indefinitely in well-aerated aquaria of artificial seawater (Instant Ocean, Aquarium Systems, Inc., Eastlake, Ohio) at 15°C. Abdominal ganglia and nerves were removed from *Aplysia* as previously described (Giller and Schwartz, 1971). Isolated nervous tissue was kept at room temperature in artificial seawater containing 50 mM tris(hydroxymethyl)-aminomethane (Tris)-HCl (pH 7.6) for 30–60 min before use.

Labeling Adenine Nucleotides

In order to measure formation of cAMP from radioactive precursors during electrical stimulation, we first incubated ganglia in adenine-^{3}H (Shimizu, Creveling, and Daly, 1970 *a*). Ganglia and nerves were pinned through connective tissue to a

silicone plastic (Sylgard, Dow Chemical Co., Midland, Mich.) in Petri dishes, covered with 50–100 μl of buffered artificial seawater containing 80 μM adenine-2-^{3}H (New England Nuclear Corp., Boston, Mass., specific activity 6–9 Ci/mmole) and incubated at room temperature. After 1 hr, the tissue was washed several times with buffered seawater. About 20% of the radioactivity was taken up into the tissue under these conditions. Each component (individual ganglion or nerve) took up between 0.48 and 1.6 × 10^6 counts per min.

Electrical Stimulation

Abdominal ganglia, which had previously been incubated in adenine-^{3}H, were pinned to paraffin in a lucite chamber at room temperature in seawater. A microelectrode was placed in cell R2 or, in some experiments, in another identified cell in the ganglion in order to monitor the effectiveness of electrical stimulation. As a result of these preparative procedures 15–20 min elapsed from the end of the incubation in adenine-^{3}H to the beginning of the period of stimulation. Peripheral nerves or connectives were stimulated in turn at a frequency of 1/sec for 1–5 min or until the postsynaptic potential (PSP) became quite small. Strong stimuli to any of these nerves initially produce large PSP's in R2 which fire the cell (Kandel and Tauc, 1965; Frazier, Kandel, Kupfermann, and Coggeshall, 1967). Ganglia were stimulated for periods of 4, 15, or 60 min. At the end of the period of stimulation the membrane potential was measured as the recording electrode was removed. It ranged between 40 and 60 mv, which is comparable to that previously recorded (see Schwartz, Castellucci, and Kandel, 1971). 3–5 min elapsed between removal of the electrode and homogenization of the ganglia. Control (unstimulated) ganglia were pinned and handled in the same way, but were not impaled with a microelectrode. Each experiment was carried out with a control matched for animal size, and selected from the same shipment of animals in order to minimize biochemical variability between animals (Schwartz et al., 1971).

Determination of cAMP-^{3}H

Connectives were removed by cutting just distal to the bag cell clusters (Frazier et al., 1967). In some experiments the cell body of R2 was dissected freehand from the ganglion in seawater (Giller and Schwartz, 1971). Ganglia and nerves were then extracted separately at 0°C in 50 μl of 0.5 M acetic acid by grinding in small glass homogenizers (Micrometrics Instrument Corp., Norcross, Ga.). Similar results were obtained when the tissue was extracted with 50% aqueous ethanol. cAMP was isolated following procedures of Shimizu et al. (1970 *a*). Extracts were centrifuged at low speed in the cold to remove particulate matter. 10- to 20-μl samples containing 5 nmoles of cAMP added as carrier were applied to plastic sheets (20 × 20 cm) coated with cellulose MN 300 impregnated with polyethyleneimine (Brinkmann Instruments, Inc., Westbury, N. Y.). More than 90% of the adenine and adenosine in the samples was removed from the sheets by immersing them in methanol for 10 min. This treatment did not remove any of the phosphorylated derivatives of adenine. cAMP was separated from the other compounds by thin-layer chromatography in *n*-butanol:ethyl acetate:methanol:concentrated ammonia

(7:4:3:4) (Shimizu et al., 1970 *a*) carried out in a sandwich chamber (Brinkmann Instruments, Inc.). In this system we found that cAMP had an R_f of 0.24; adenine, 0.39; and adenosine, 0.51. ATP, adenosine diphosphate (ADP), AMP, and polynucleotides remained at the origin. After locating the position of cAMP in ultraviolet light, we scraped the cellulose off the plate into 1 ml of 0.01 N HCl in order to extract cAMP selectively. The cellulose was removed by centrifugation, the extract neutralized with NaOH, and cAMP was purified by the addition of $ZnSO_4$ and $Ba(OH)_2$, which precipitated residual contaminants (Krishna, Weiss, and Brody, 1968). After centrifugation, the supernatant containing cAMP was counted by scintillation. The overall recovery of cAMP was 70–80% and was determined by internal standardization for each sample by measuring the recovery of ultraviolet absorbance of cAMP added initially as carrier. Values for formation of cAMP in nervous tissue were corrected for recovery, and are expressed as per cent of total radioactivity taken up by the tissue.

Measurements of Total cAMP

Extracts of neural tissue and individual cell bodies in 50% aqueous ethanol were evaporated to dryness under a stream of nitrogen gas and assayed for cAMP by isotope displacement exactly as described by Gilman (1970).

Identification of Nucleotides

Radioactivity identified as cAMP was eluted from the polyethyleneimine plates with 0.5 M acetic acid. After the cellulose was removed, the extracts were evaporated to dryness and incubated at 30°C for 30 min with 0.44 U of 3′,5′ cyclic nucleotide phosphodiesterase from beef heart (Sigma Chemical Co., St. Louis, Mo.) in 50 μl of 2 mM $MgCl_2$, 20 mM Tris-HCl (pH 7.5) with 5 μg of cAMP added as carrier. Radioactivity remaining in solution after the addition of $ZnSO_4$ and $Ba(OH)_2$ was counted by liquid scintillation.

ATP in extracts of neural tissue was assayed with firefly luciferase (Strehler, 1965) using a scintillation counter to monitor appearance of light. With the crude firefly extract used (Worthington Biochemical Corp., Freehold, N. J.) ADP would also contribute to the values obtained. Distribution of radioactive nucleotides in tissue extracts was determined by paper electrophoresis at pH 4.4 in 0.05 M sodium citrate buffer for 90 min in a potential gradient of 33 v/cm on a water-cooled copper plate (Savant Instruments, Hicksville, N. Y.).

These experiments were done from November to May.

RESULTS

Increased cAMP after Electrical Stimulation

Stimulation of either peripheral nerves or connectives of the abdominal ganglion for 15 min doubled the amount of cAMP formed from adenine-^{3}H (Table I, A, 2). The difference between stimulated and control ganglia was significant ($P < 0.005$) using a two-tailed t test. Intracellular electrophysiological records were obtained from identified neurons (usually R2) in all

TABLE I

INCREASED FORMATION OF cAMP-^{3}H AS A RESULT OF ELECTRICAL STIMULATION

	cAMP	
	Control	Stimulated
	% of total radioactivity/kg	
A. Duration of stimulation		
1. 4 min	0.9±0.1 (2)	0.8±0.1 (2)
2. 15 min	0.8±0.1 (18)	1.6±0.1 (15)
3. 60 min	0.7±0.1 (4)	1.4±0.2 (4)
B. Nerves (15 min of stimulation)	0.9±0.1 (6)	0.9±0.1 (6)
C. Conditions affecting formation		
1. Artificial seawater + theophylline (1 mM)	0.7±0 (2)	1.5±0.1 (2)
2. Complete salts with 200 mM Mg^{2+}	0.7±0.1 (7)	0.6±0.1 (7)

Mean values for all measurements are presented ± SEM of (n) determinations.

Nervous tissue was incubated in adenine-^{3}H for 1 hr before the stimulation was begun (see Methods). Formation of cAMP is presented as a per cent of the radioactivity taken up into the tissue converted to cAMP-^{3}H/weight of animal (kg). A. Abdominal ganglia were used for these experiments. 1. The animals weighed 60, 75 (control), 85, 90 g (stimulated). 2. Animals ranged from 40 to 180 g with an average weight of 108 g. The values for cAMP obtained before normalization by animal weight were: (control) 0.06 ± 0.01; (stimulated) 0.12 ± 0.02. 3. Animals weighed 80–150 g with an average of 120 g. B. Nerves removed from some of the ganglia in experiment A 2. C. Abdominal ganglia were stimulated electrically for 15 min. 1. Theophylline was present from the end of the labeling period until the end of stimulation. The average animal weight was 180 g. 2. Complete salts solution with added Mg^{2+} contained (millimoles/liter) NaCl, 425; KCl, 10; $CaCl_2$, 10.23; $MgCl_2$, 174; $NaHCO_3$, 2.8; $MgSO_4$, 26; and Tris-HCl (pH 7.6), 50. Ganglia from animals with an average weight of 160 g were bathed in this medium from the end of the labeling period until the end of stimulation (about 40 min).

stimulated ganglia in order to monitor the effectiveness of synaptic input. The stimulus strength was adjusted to produce large excitatory PSP's which were capable of eliciting action potentials in the monitored cell. One typical recording of the input to R2 is presented in Fig. 1. Although we have not examined this point systematically, connectives and peripheral nerves seemed equally capable of producing the increase. To examine how duration of stimulation affected cAMP, we also stimulated ganglia for periods of 4 and 60 min. No detectable increase was obtained after a period of only 4 min. Prolonging the period of stimulation to 1 hr did not enhance the extent of the effect (Table I, A, 1 and 3). We have not determined why continued stimulation did not produce further increases in cAMP.

These changes were limited to the ganglion. Electrical stimulation did not cause increased formation of cAMP-^{3}H within the nerves or connectives themselves (Table I, B). This failure was not the result of a difference between nerves and ganglia in the specific radioactivity of ATP. ATP in both structures had similar specific activities. Moreover, as is shown in the accompanying paper (Cedar and Schwartz, 1972), the synthesis of cAMP in response to the

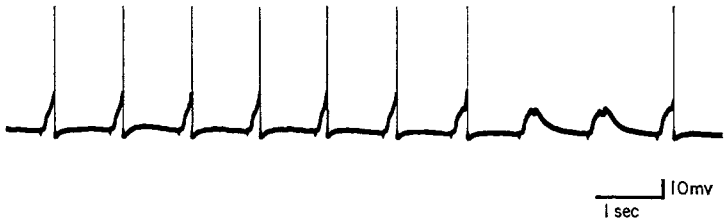

FIGURE 1. Response of R2 to synaptic stimulation. The left connective was stimulated at 1/sec in order to produce excitatory postsynaptic potentials which were usually of sufficient magnitude to initiate action potentials. The occasional failure of spike generation reveals the configuration of the complex excitatory postsynaptic potential.

application of serotonin was greater in connectives than in ganglia. We were not able to localize further the site of the increase because we could not detect reliable changes even in the largest identified nerve cell bodies (R2 and R15).

The greater amount of radioactivity found in cAMP after stimulation resulted from an enhanced rate of synthesis rather than from an increase in the specific radioactivity of its precursor, ATP, or of other adenine-containing compounds. Maitra, Ghosh, Schoener, and Chance (1964), in the electroplax, and Giacobini and Grosso (1966), in the stretch receptor neuron of the crayfish, using schedules of stimulation different from ours, reported that impulse activity diminished the concentration of ATP. In *Aplysia* both the proportion of the total radioactivity in the form of ATP and the amount of total ATP present in the ganglion were unaffected by incubation in 80 μM adenine and were unaltered under our conditions of electrical stimulation (Table II). The amount of total radioactivity found in stimulated ganglia remained the same as that taken up into control ganglia. Furthermore, the distribution of other labeled compounds formed from adenine-^{3}H was identical in both control and stimulated ganglia (11 determinations of each type). In addition to ATP, the percentages of other compounds in ganglion extracts in the 22 experiments were: ADP, 19.8 $\pm$ 1.6, AMP, 13.1 $\pm$ 1, and adenine, 0.8 $\pm$ 0.06. Adenosine and polynucleotides, which remained at the origin, were unresolved by high voltage electrophoresis; together they amounted to 15.4 $\pm$ 1.2%.

Although the standard isolation of cAMP involved several purification procedures, we tested the material further by subjecting it to digestion with beef heart 3′,5′ cyclic nucleotide phosphodiesterase. Radioactivity isolated as cAMP from both electrically stimulated and control ganglia was almost quantitatively hydrolyzed, demonstrating that it was truly cAMP.

In contrast to the increase in synthesis of cAMP from adenine-^{3}H, there was no detectable effect of electrical stimulation on the *total content* of cAMP either in the whole ganglion or in the cell body of R2 isolated by microdissection (Table III). Thus the stimulation appears to affect a restricted fraction of the cAMP in the ganglion, which, possibly by virtue of its localization in specific

TABLE II

CONTENT OF ATP IN CONTROL ABDOMINAL GANGLIA AND IN GANGLIA STIMULATED ELECTRICALLY FOR 15 MIN

Determination	Control	Stimulated
Total ATP, *nmoles/ganglion*	11.8±2 (5)	11.2±2 (5)
ATP-^{3}H, *% of total radioactivity*	51.5±4 (11)	50.2±4 (11)

Mean values for all measurements are presented ± SEM of (n) determinations.
Total ATP was assayed (see Methods) in abdominal ganglia from *Aplysia* weighing 90–150 g with an average weight of 120 g. Similar ganglia incubated for 1 hr in 80 μM adenine had 10.1 ± 2 (4) nmoles of ATP. *ATP-^{3}H* was measured in extracts of ganglia previously incubated with adenine-^{3}H from animals weighing 40–100 g with an average weight of 81 g.

TABLE III

TOTAL cAMP IN THE ABDOMINAL GANGLION AND IN THE CELL BODY OF THE GIANT NEURON, R2, AFTER A 15 MIN PERIOD OF ELECTRICAL STIMULATION

Neural component	cAMP	
	Control	Stimulated
	pmoles	
Abdominal ganglion	9.1±1.1 (5)	8.4±0.9 (5)
Cell bodies of R2	0.6±0.1 (4)	0.6±0.2 (4)

Mean values for all measurements are presented ± SEM of (n) determinations.
Cell bodies were dissected from the ganglia within 4 min after the period of stimulation. Total cAMP was measured by isotope displacement (Gilman, 1970). *Aplysia* weighed 90–150 g with an average weight of 120 g.

neurons, parts of neurons, or in glia, either turns over more rapidly than does the total cAMP of the ganglion, or is synthesized from ATP with a considerably higher specific radioactivity than that of the total ATP of the ganglion. These results differed from those obtained in the sympathetic cervical ganglion, where McAfee et al. (1971) found large increases in total cAMP after preganglionic stimulation. Large increases, however, may not be required for cAMP to produce its total effect. In many tissues only a small increment of cAMP is enough to produce maximal physiological activity, whereas in response to the application of various hormones, the possible increase in cAMP content is considerably greater (Robinson, Butcher, and Sutherland, 1971, see especially p. 294).

It can be estimated from values for total cAMP in the ganglion presented in Table III, and values for the amount formed from adenine-^{3}H during 1 hr, that the amount of cAMP labeled in control ganglia was about 5% of the

total cAMP present.[1] A similar proportion of labeled to total cAMP was reported by Shimizu et al. (1970 *a*) in brain slices of the guinea pig.

Conditions Affecting the Increase in cAMP

In the accompanying paper Cedar and Schwartz describe how the content of cAMP in ganglia varies with animal weight, increasing by approximately 50% for each 100 g increment in the mass of the animal. The elevation of cAMP brought about by electrical stimulation did not appear to vary with the weight of the animal (Table I).

Addition to the bath of the phosphodiesterase inhibitor, theophylline, did not affect the amount of cAMP formed either in electrically stimulated or in control ganglia (Table I, C, 1). Nevertheless, we found that theophylline was effective in protecting cAMP synthesized in extracts of *Aplysia* nervous tissue and also enhanced accumulation of cAMP in intact *Aplysia* nervous tissue brought about by the application of serotonin (Cedar and Schwartz, 1972).

To test whether accumulation of cAMP in the ganglion depended upon activity at chemical synapses, we blocked chemical transmission by raising the concentration of Mg^{2+} to 200 mM, 4 times the normal concentration. High concentrations of Mg^{2+} ion have been shown to inhibit release of transmitter substances at other chemical synapses (del Castillio and Katz, 1954). We recorded in R2 while stimulating peripheral nerves and connectives in all of these experiments, and confirmed observations made previously that 200 mM Mg^{2+} ion blocks synaptic transmission in the abdominal ganglion of *Aplysia* (Castellucci, Pinsker, Kupfermann, and Kandel, 1970; Kupfermann, Pinsker, Castellucci, and Kandel, 1971). We found that high concentrations of Mg^{2+} also blocked the effect of electrical stimulation on the enhanced production of cAMP (Table I, C, 2). This inhibition is not a direct effect on the synthesis of cAMP since intact, isolated nervous tissue of *Aplysia* is capable of elevated formation of cAMP in the presence of this high concentration of Mg^{2+} ion (Cedar and Schwartz, 1972). The elevated concentration of Mg^{2+} ion also did not interfere with conduction of nerve impulses; directly and antidromically initiated spikes could still be reliably evoked.

Inhibition by Mg^{2+} is therefore consistent with the notion that impulse activity in presynaptic neurons was by itself insufficient to bring about an elevation of cAMP. Also consistent with this idea was our observation that ouabain and glutamate did not cause increases in cAMP. At a concentration of 0.2 mM,

[1] Radioactivity taken up by an abdominal ganglion was calculated to be 800 pmoles using the nominal concentration of adenine-^{3}H. The per cent of total radioactivity in the form of cAMP was 0.06 (see legend to Table I, A, 2), which was equivalent to 0.48 pmoles. Since total cAMP was 9.1 pmoles/ganglion (Table III) labeled cAMP was approximately 5% of the total. Using a similar calculation we determined that 5–10% of the ATP was labeled.

glutamate depolarized *Aplysia* neurons; thus R2, normally a silent cell, fired repetitively for several hours in the presence of the amino acid (Koike, unpublished experiments). Application of glutamate had no effect on the cAMP in *Aplysia* nervous tissue (Cedar and Schwartz, 1972). In five experiments, we also tested 0.1 mM ouabain, applied for 7 min, which rapidly depolarized most, and perhaps all, neurons in *Aplysia*, and caused them to fire repeatedly (Carpenter and Alving, 1968; Pinsker and Kandel, 1969). Both glutamate and ouabain caused transient increases in spontaneous PSP's in the ganglion because of their depolarizing actions on interneurons. This synaptic activity did not affect cAMP, possibly because it was too weak or too transient. In vertebrate brain slices adenosine is thought to be released after depolarization brought about either by electrical shocks or pharmacologically, for example by ouabain; adenosine and the adenine nucleotides have been shown to stimulate the formation of cAMP (Sattin and Rall, 1970; Shimizu et al., 1970 *a*, *b*). Nevertheless, in *Aplysia* adenosine was ineffective at a concentration of 0.2 mM (three experiments).

DISCUSSION

In nervous tissue two quite different processes, impulse activity and synaptic activity, might initiate changes in cAMP. McAfee et al. (1971), using the superior cervical ganglion of the rabbit, and we, using the abdominal ganglion of *Aplysia*, have presented evidence that synthesis of cAMP in a ganglion can vary in response to synaptic input. Our results do not permit us to conclude definitely that the changes in cAMP take place in neurons; they may be localized to other elements, for example, to glia. Whatever the localization of these changes in cAMP, the following observations suggest to us that they resulted from synaptic stimulation rather than from impulse activity.

(*a*) The nerves of the ganglion did not respond with increased amounts of cAMP, even though they were known to be conducting impulses. It can be shown that nerves are fully capable of increasing cAMP, and do so in response to the application of serotonin and dopamine (Cedar and Schwartz, 1972). (*b*) The increase in cAMP produced by synaptic stimulation was blocked by an elevated concentration of Mg^{2+} ion, which blocked synaptic activity in the ganglion without impairing conduction of impulses. This is not likely to be a direct effect of Mg^{2+} on the synthesis of cAMP, since the increase in cAMP resulting from the application of serotonin, which is greater than that observed with electrical stimulation, was not at all influenced by this concentration of Mg^{2+} ion (Cedar and Schwartz, 1972). (*c*) Depolarization produced by ouabain (Carpenter and Alving, 1968; Pinsker and Kandel, 1969), and glutamate (H. Koike, personal communication), which results in increased rates of firing, did not cause accumulation of cAMP. Thus, increased formation of cAMP in

the abdominal ganglion after stimulation of nerves and connectives seems to result from a mechanism involving synaptic stimulation.

We tentatively propose that one or more transmitter substances which are released by fibers afferent to the ganglion, and which bring about changes in membrane permeability, also initiate increases in cAMP postsynaptically after electrical stimulation. Siggins, Hoffer, and Bloom (1969) have presented evidence that cAMP itself might be essential for production of the synaptic potentials which occur in cerebellar Purkinje cells as a consequence of adrenergic transmission. Our observation that electrical stimulation in *Aplysia* resulted in a relatively prolonged elevation of cAMP might suggest an additional function. In the abdominal and the buccal ganglion of *Aplysia* a single transmitter, acetylcholine, is capable of mediating excitation to some cells, inhibition to others, and dual excitation-inhibition to a third class of neurons (Kandel, Frazier, Waziri, and Coggeshall, 1967; Wachtel and Kandel, 1971; Gardner and Kandel, 1972). The existence of many transmitter substances is therefore not essential for varied synaptic function, since, in principle, a single substance could mediate synaptic activity of any sign. Perhaps nervous systems make use of more than one transmitter substance because each may have different biochemical consequences.

Some support for this idea is provided in the accompanying paper (Cedar and Schwartz, 1972) in which it is shown that serotonin and dopamine, but not carbachol, glutamate, or norepinephrine, produced marked increases in cAMP in *Aplysia* nervous tissue. A particularly interesting example of specificity is the recent observation that acetylcholine increased cyclic guanosine monophosphate (cGMP), but not cAMP, in vertebrate brain and heart muscle, two tissues in which cAMP is greatly enhanced by catecholamines (George, Polson, O'Toole, and Goldberg, 1970; Ferrendelli, Steiner, McDougal, and Kipnis, 1970; Kuo, Lee, Reyes, Walton, Donnelly, and Greengard, 1972). At the present time, there is not enough information available to determine whether all metabolic effects of synaptic activity are mediated by these two cyclic nucleotides. By examining the metabolic consequences of synaptic activity on different neurons, it may be possible to test whether each transmitter might mediate relatively specific kinds of biochemical processes in the postsynaptic neuron, in addition to a family of common synaptic actions.

We thank Doctors Irving Kupfermann and W. Alden Spencer for reading the manuscript critically. This research was supported by U. S. Public Health Service Career Scientist Award MH 18,558 to Dr. E. R. Kandel and Career Award GM-28,125 to Dr. J. H. Schwartz, as well as by National Institutes of Health Research Grants NS 09361, NS 08159, and MH 19795, and National Science Foundation Grant GB-25046.

Received for publication 19 April 1972.

REFERENCES

BERRY, R. W. 1969. Ribonucleic acid metabolism of a single neuron: correlation with electrical activity. *Science* (*Wash. D. C.*). **166**:1021.

CARPENTER, D. O., and B. O. ALVING. 1968. A contribution of an electrogenic Na^+ pump to membrane potential in *Aplysia* neurons. *J. Gen. Physiol.* **52**:1.

CASTELLUCCI, V., H. PINSKER, I. KUPFERMANN, and E. R. KANDEL. 1970. Neuronal mechanisms of habituation and dishabituation of the gill withdrawal reflex in *Aplysia*. *Science* (*Wash. D. C.*). **167**:1745.

CEDAR, H., and J. H. SCHWARTZ. 1972. Cyclic adenosine monophosphate in the nervous system of *Aplysia californica*. II. Effect of serotonin and dopamine. *J. Gen. Physiol.* **60**:570.

DEL CASTILLIO, J., and B. KATZ. 1954. The effects of magnesium on the activity of motor nerve endings. *J. Physiol.* (*Lond.*). **124**:553.

FERRENDELLI, J. A., A. L. STEINER, D. B. MCDOUGAL, JR., and D. M. KIPNIS. 1970. The effect of oxotremorine and atropine on cGMP and cAMP levels in mouse cerebral cortex and cerebellum. *Biochem. Biophys. Res. Commun.* **41**:1061.

FRAZIER, W. T., E. R. KANDEL, I. KUPFERMANN, and R. E. COGGESHALL. 1967. Morphological and functional properties of identified neurons in the abdominal ganglion of *Aplysia californica*. *J. Neurophysiol.* **30**:1288.

GARDNER, D., and E. R. KANDEL. 1972. Diphasic postsynaptic potential: a chemical synapse capable of mediating conjoint excitation and inhibition. *Science* (*Wash. D. C.*). **176**:675.

GEORGE, W. J., J. B. POLSON, A. G. O'TOOLE, and N. D. GOLDBERG. 1970. Elevation of guanosine 3′,5′-cyclic phosphate in rat heart after perfusion with acetylcholine. *Proc. Natl. Acad. Sci.* (*U.S.A.*) **66**:398.

GERSCHENFELD, H. M. 1966. Chemical transmitters in invertebrate nervous systems. *Symp. Soc. Exp. Biol.* **20**:299.

GIACOBINI, E., and A. GROSSO. 1966. Variations of glycolytic intermediates, phosphate compounds and pyrimidine nucleotides after prolonged stimulation of an isolated crustacean neurone. *Acta Physiol. Scand.* **66**:49.

GILLER, E., and J. H. SCHWARTZ. 1971. Choline acetyltransferase in identified neurons of abdominal ganglion of *Aplysia californica*. *J. Neurophysiol.* **34**:93.

GILMAN, A. G. 1970. A protein binding assay for adenosine 3′,5′-cyclic monophosphate. *Proc. Natl. Acad. Sci.* (*U.S.A.*) **67**:305.

GREENGARD, P., and E. COSTA, editors. 1970. Role of Cyclic AMP in Cell Function. Raven Press, New York.

KAKIUCHI, S., and T. W. RALL. 1968 *a*. The influence of chemical agents on the accumulation of adenosine 3′,5′-phosphate in slices of rabbit cerebellum. *Mol. Pharmacol.* **4**:367.

KAKIUCHI, S., and T. W. RALL. 1968 *b*. Studies on adenosine 3′,5′-phosphate in rabbit cerebral cortex. *Mol. Pharmacol.* **4**:379.

KAKIUCHI, S., T. W. RALL, and H. MCILWAIN. 1969. The effect of electrical stimulation upon the accumulation of adenosine 3′,5′-phosphate in isolated cerebral tissue. *J. Neurochem.* **16**:485.

KANDEL, E. R., W. T. FRAZIER, R. WAZIRI, and R. E. COGGESHALL. 1967. Direct and common connections among the identified neurons in *Aplysia*. *J. Neurophysiol.* **30**:1352.

KANDEL, E. R., and L. TAUC. 1965. Heterosynaptic facilitation in neurones of the abdominal ganglion of *Aplysia depilans*. *J. Physiol.* (*Lond.*). **181**:1.

KERNELL, D., and R. P. PETERSON. 1970. Effects of spike activity versus synaptic activation on metabolism of ribonucleic acid in a molluscan giant neuron. *J. Neurochem.* **17**:1087.

KRISHNA, G., B. WEISS, and B. B. BRODY. 1968. A simple, sensitive method for the assay of adenyl cyclase. *J. Pharmacol. Exp. Ther.* **163**:379.

KUO, J. -F., T. P. LEE, P. L. REYES, K. G. WALTON, T. E. DONNELLY, JR., and P. GREENGARD. 1972. Cyclic nucleotide-dependent protein kinase. X. An assay method for the measurement of guanosine 3′,5′-monophosphate in various biological materials and a study of agents regulating its levels in heart and brain. *J. Biol. Chem.* **247**:16.

KUPFERMANN, I., H. PINSKER, V. CASTELLUCCI, and E. R. KANDEL. 1971. Central and peripheral control of gill movements in *Aplysia*. *Science* (*Wash. D. C.*). **174**:1252.

LARABEE, M. G., and W. S. LEICHT. 1965. Metabolism of phosphatidyl inositol and other lipids in actual neurones of sympathetic ganglia and other peripheral nervous tissues. The site of the inositide effect. *J. Neurochem.* **12**:1.

MAITRA, P. K., A. GHOSH, B. SCHOENER, and B. CHANCE. 1964. Transients in glycolytic metabolism following electrical activity in electrophorus. *Biochim. Biophys. Acta.* **88**:112.

MCAFEE, D. A., M. SCHORDERET, and P. GREENGARD. 1971. Adenosine 3′,5′-monophosphate in nervous tissue: increase associated with synaptic transmission. *Science* (*Wash. D. C.*). **171**:1156.

PETERSON, R. P., and D. KERNELL. 1970. Effects of nerve stimulation on metabolism of ribonucleic acid in a molluscan giant neurone. *J. Neurochem.* **17**:1075.

PINSKER, H., and E. R. KANDEL. 1969. Synaptic activation of an electrogenic sodium pump. *Science* (*Wash. D. C.*). **163**:931.

ROBISON, G. A., R. W. BUTCHER, and E. W. SUTHERLAND. 1971. Cyclic AMP. Academic Press Inc., New York.

SATTIN, A., and T. W. RALL. 1970. The effect of adenosine and adenine nucleotides on the cyclic adenosine 3′,5′-phosphate content of guinea pig cerebral cortex slices. *Mol. Pharmacol.* **6**:13.

SCHWARTZ, J. H., V. F. CASTELLUCCI, and E. R. KANDEL. 1971. Functioning of identified neurons and synapses in abdominal ganglion of *Aplysia* in absence of protein synthesis. *J. Neurophysiol.* **34**:939.

SHIMIZU, H., C. R. CREVELING, and J. DALY. 1970 *a*. Stimulated formation of adenosine 3′,5′-cyclic phosphate in cerebral cortex: synergism between electrical activity and biogenic amines. *Proc. Natl. Acad. Sci.* (*U.S.A.*) **65**:1033.

SHIMIZU, H., C. R. CREVELING, and J. W. DALY. 1970 *b*. Cyclic adenosine 3′,5′-monophosphate formation in brain slices: stimulation by batrachotoxin, ouabain, veratridine, and potassium ions. *Mol. Pharmacol.* **6**:184.

SIGGINS, G. R., B. J. HOFFER, and F. E. BLOOM. 1969. Cyclic adenosine monophosphate: possible mediator for norepinephrine effects on cerebellar Purkinje cells. *Science* (*Wash. D. C.*). **165**:1018.

STREHLER, B. L. 1965. Adenosine-5′-triphosphate and creatine phosphate. Determination with luciferase. *In* Methods of Enzymatic Analysis. H.-V. Bergmeyer, editor. Academic Press Inc., New York. 559.

TAUC, L. 1967. Transmission in invertebrate and vertebrate ganglia. *Physiol. Rev.* **47**:521.

WACHTEL, H., and E. R. KANDEL. 1971. Conversion of synaptic excitation to inhibition at a dual chemical synapse. *J. Neurophysiol.* **34**:56.