cultured flagellates were examined by electron microscopy (*10*) to determine whether antibiotic treatment had rendered them endosymbiote-free. No endosymbiotic bacteria were ever observed in these flagellates. They possessed food vacuoles containing cellulose particles and heat-killed food bacteria (Fig. 1b), along with the usual cell organelles present in this and related hypermastigotes (*14, 15*). Since no living bacteria were present in the culture fluid nor within the flagellates, the cultures were axenic. *T. sphaerica* has since been grown in continuous axenic culture for more than 15 months.

Cellulose was required for growth by axenic *T. sphaerica* which suggested that the flagellates possessed enzymes for cellulose metabolism. The end products of cellulose metabolism were determined by growing axenic flagellates in medium containing labeled cellulose (*16*). The results after several weeks of incubation with ^{14}C-labeled cellulose showed that *T. sphaerica* produced CO_2 and acetate, which accounted for about 30 and 58 percent of the label released into the culture fluid, respectively (*17*). Hydrogen was also produced by the flagellates (*18*). These end products are the same as those produced by axenic *Trichomitopsis termopsidis* (*16*) and by *Trichonympha* spp. from *Zootermopsis* (*3*).

These results indicate that *T. sphaerica* is itself capable of cellulose metabolism without the participation of endosymbiotic bacteria. The axenic flagellates metabolized cellulose intracellularly and released acetate, a substrate that would be absorbed from the termite intestine and oxidized (*3*) or used in biosynthesis (*19*). Although numerous symbiotic associations between bacteria and hypermastigote flagellates have been reported (*5, 14*), the assumed role of endosymbiotes in cellulose metabolism by these flagellates should be viewed with skepticism in view of the data presented above.

Hypermastigote flagellates display many interesting characteristics that can now be studied in vitro. They are obligate anaerobes with unusual metabolism (*2, 3*); they ferment cellulose to potentially useful end products, have complex ultrastructures (*14, 15*) and an unusual reproductive process (*8*), and some undergo sexual cycles in response to molting of the host (*20*). These highly evolved organisms should no longer be regarded as interesting but recalcitrant members of the biological world.

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11. These were autoclaved washed rumen bacteria and had no cellulolytic activity [M. A. Yamin and W. Trager, *J. Gen. Microbiol.* **113**, 417 (1979)].
12. These termites harbored *T. sphaerica* as the only hypermastigote present.
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16. End products released into the culture fluid were quantified and identified as described [M. A. Yamin, *Appl. Environ. Microbiol.* **39**, 859 (1980)]. Briefly, labeled CO_2 was trapped in phenylethylamine from acidified culture fluid. Labeled acetate was separated and quantified by vacuum distillation and ether extraction; it was identified by thin-layer chromatography and by a substrate-specific reaction with acetate kinase.
17. Average of two determinations on two 7-week-old cultures. Metabolism of labeled cellulose was very slow, but analysis of end products at earlier times gave similar results.
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Effect of Adenosine 3′,5′-Monophosphate on Neuronal Pacemaker Activity: A Voltage Clamp Analysis

Abstract. *Bursting pacemaker activity in nerve cells can be modified for long periods by synaptic input of short duration. There is evidence that cyclic nucleotides may play a role in these modifications. The predominant effect of elevated levels of adenosine 3′,5′-monophosphate in* Aplysia *neurons was an increased slope conductance to hyperpolarizing pulses, evident in voltage clamp records. A similar increase in slope conductance was seen as one component of maximum strength synaptic stimulation, which is consistent with the idea that cyclic nucleotides are important in the expression of synaptic alteration of bursting pacemaker activity.*

It has been suggested that cyclic nucleotides function as "second messengers" in a number of synaptic events in neural systems. An analysis of the role of cyclic nucleotides in these events has been lacking because of difficulties inherent in obtaining biophysical measurements of membrane responses in complex neural tissue. We used a neuron from the nervous system of the marine mollusk *Aplysia* to perform such an analysis. Cell R15 of *Aplysia* is subject to long-lasting hyperpolarization after brief stimulation of the branchial nerve (*1*). This synaptic response is enhanced in the presence of theophylline (*2*), a phosphodiesterase inhibitor thought to prolong the actions of cyclic nucleotides by preventing their breakdown. Phosphodiesterase inhibitors may have effects on processes other than those related to cyclic nucleotide action. Additional evidence of cyclic nucleotide involvement is the finding that intracellular injection of 5′-guanylyl imidodiphosphate (GMP-PNP), which activates adenylate cyclase, causing increased synthesis of adenosine 3′,5′-monophosphate (cyclic AMP), produces a long-lasting hyperpolarization of cell R15 (*2*).

In our study we use voltage clamp techniques to elucidate the pseudo-steady-state current-voltage (*I-V*) relationship in R15 during nucleotide action. The *I-V* curve induced by this action is compared with that produced by synaptic stimulation. Our analysis serves both to examine the manner in which cyclic AMP exerts its effects and to further test the hypothesis that long-lasting synaptic events are mediated by cyclic nucleotides. The biophysical events must be similar if cyclic nucleotides are mediating the synaptic event.

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Abdominal ganglia from *Aplysia californica* (150 to 200 g) were removed from the animal and pinned out in a Sylgard-lined dish. Cell R15 was exposed by microdissection of the overlying connective tissue sheath. Voltage clamping was by conventional means in which separate voltage-sensing and current-passing micropipette electrodes were used. The cell was held at a potential of −60 mV and was clamped to test voltages for 3 seconds. Current was measured at the end of the pulse. Intracellular injection was performed with a partitioned theta-glass pipette, as described (*3*). One-half of this pipette served as the voltage electrode of the clamp circuit and the other as the injection barrel. Injection was by positive pressure.

Cell R15 is characterized by stereotypical activity in which bursts of action potentials are separated by interburst hyperpolarizations. The mechanisms of this burst activity have received intensive examination, and the presence of a negative slope resistance (NSR) region in the pseudo-steady-state *I-V* plot appears to play an important role in the generation of bursting (*4*). It has been shown that both bursting activity and NSR may be blocked by iontophoresis of certain neurotransmitters (*5*). Figure 1 demonstrates the typical effect of an injection of 8-benzylthioadenosine 3′,5′-monophosphate into cell R15. The primary effect is an enhancement of the hyperpolarization that separates action potential bursts (Fig. 1A). In a number of cases, the cell was completely silenced. After the injection the *I-V* curve shows a prominent increase in the slope conductance, which is most evident in the more hyperpolarizing steps. The NSR is not greatly affected. The potential at which the curves cross lies very near to the computed equilibrium potential for potassium (*6*). The increased slope caused by injection was shifted rightward in a solution with an elevated level of potassium, which suggests that the primary effect of injecting cyclic AMP is to increase potassium conductance. This would produce the hyperpolarization of the cell.

Figure 1B illustrates the typical effect of intracellular injection of GMP-PNP. In this case, the cell is progressively hyperpolarized and finally silenced. The *I-V* plot of the cell is characterized by a large increase in the slope conductance, evident in response to hyperpolarizing pulses. As in the case of cyclic AMP injection, the curve is shifted to the right in the presence of elevated potassium, suggesting that a potassium conductance is predominant. The NSR is still present and, as would be expected, when an injected cell is unclamped and depolarized by current injection, bursting is reinstated (Fig. 1C). Very large injections of GMP-PNP or measurements made at later times after injection cause both an increased slope conductance and a loss of NSR; we cannot rule out a direct effect on the NSR under these conditions. However, it is clear that silencing of R15 by cyclic AMP is always accompanied by an increase in slope conductance to potassium, presumably as the lowest threshold and most specific response. An increased slope conductance was also produced when cyclic AMP itself was injected into R15 in ganglia that had been treated previously with the phosphodiesterase inhibitor RO 20-1724 (Hoffmann-La Roche). Injection of lower concentrations of GMP-PNP or of buffered distilled water did not elicit the characteristic changes described above, making it unlikely that the observed changes were due simply to an injection artifact resulting from cell damage.

Alteration of the *I-V* plot of R15 after maximum stimulation of the branchial nerve, sufficient to silence the cell for many minutes, is shown in Fig. 2B. Two

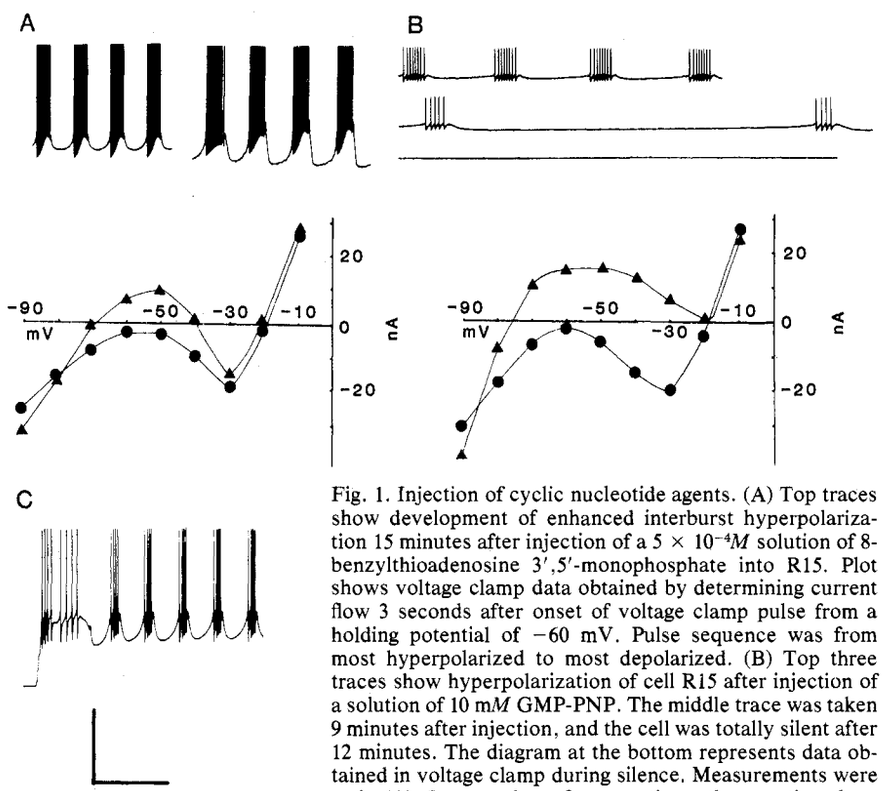

Fig. 1. Injection of cyclic nucleotide agents. (A) Top traces show development of enhanced interburst hyperpolarization 15 minutes after injection of a $5 \times 10^{-4}M$ solution of 8-benzylthioadenosine 3′,5′-monophosphate into R15. Plot shows voltage clamp data obtained by determining current flow 3 seconds after onset of voltage clamp pulse from a holding potential of −60 mV. Pulse sequence was from most hyperpolarized to most depolarized. (B) Top three traces show hyperpolarization of cell R15 after injection of a solution of 10 m*M* GMP-PNP. The middle trace was taken 9 minutes after injection, and the cell was totally silent after 12 minutes. The diagram at the bottom represents data obtained in voltage clamp during silence. Measurements were as in (A). In a number of preparations, the negative slope resistance (NSR) region dipped into the inward current range, even after injection. (C) Voltage trace of a different R15 completely silent after injection of GMP-PNP. Depolarizing current induces a sequence of bursting activity, consistent with the continued presence of an NSR. Tops of spikes are clipped by penwriter in all cases. Calibrations: (A) 60 mV, 40 seconds; (B) 150 mV, 8 seconds; and (C) 60 mV, 8 seconds. Symbols: (A) and (B) circles, controls; triangles, injected cells.

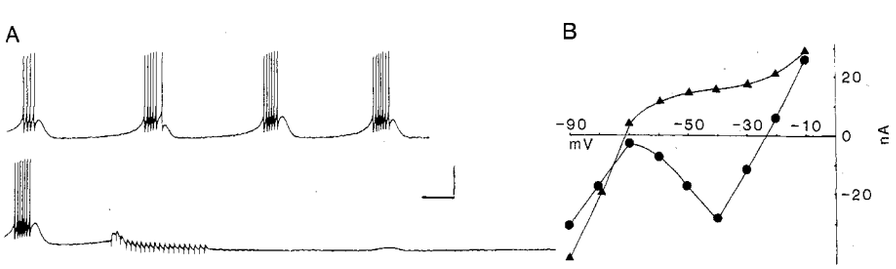

Fig. 2. Result of branchial nerve stimulation. (A) Top trace shows control, and bottom trace shows silencing of cell after 20 stimuli, 0.5 msec each, to branchial nerve through suction electrode. (B) Plot of current versus voltage in voltage clamp. Measurements were as in Fig. 1A, and pulse paradigm was begun 30 seconds after termination of branchial nerve stimulation. Calibration: 30 mV, 48 seconds. Symbols: circles, control; triangles, branchial nerve stimulation.

effects that are evident are an increased slope conductance at very hyperpolarized membrane potentials and a significant decrease in the NSR. Although some of the reduction of NSR may result from a competing potassium conductance, both of these effects can be produced independently by varying stimulus parameters.

Our results are most consistent with a cyclic AMP mediation of those inputs that act mainly via an increased slope conductance, presumed to be potassium-mediated. A more mechanistic analysis of the mediation of these long-lasting synaptic events in R15 awaits a more detailed description of the conductance changes evoked by individual fibers within the branchial nerve. Bursting pacemaker activity is an important feature of normal neuronal activity; it is also an important feature of abnormal activity, possibly manifested in disorders such as epilepsy. The finding that the conductances underlying such activity may be under synaptic control suggests that complex output patterns may be produced or altered by relatively short input regimes.

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Lactose Facilitates the Intestinal Absorption of Lead in Weanling Rats

Abstract. *The milk sugar lactose is known to facilitate calcium absorption and has been shown to enhance the uptake of essential trace metals from the intestines as well. Its physiological role as the major carbohydrate source for suckling mammals is thus complemented by its ability to facilitate the absorption of necessary minerals. The studies reported here show that the intestinal absorption of lead and its uptake into blood, liver, kidney, and bone are also increased by lactose in young weanling rats. These data extend the known range of lactose facilitation of mineral absorption to a nonessential, toxic element, confirming the nonspecificity of its action on the gut. In addition, they suggest an explanation for some of the conflicting evidence regarding the prophylactic efficacy of milk in lead poisoning.*

It is well known that children are more susceptible than adults to lead poisoning (*1*). This sensitivity to the toxicity of lead results in part from increased absorption and retention of ingested lead (*2*). Enhanced absorption of lead by the young is probably a general characteristic of mammals and has been documented in nonhuman primates (*3*) and in rodents (*4*). The reasons for the subsequent decline in lead retention with age are not completely understood but may include the development of more selective intestinal absorptive processes, more efficient biliary and renal excretion of absorbed lead, and changes in the diet.

Dietary changes may be particularly significant in mammals, since the preweaning diet under normal circumstances consists almost exclusively of mother's milk, whereas the postweaning diet contains little or no milk. Milk diets have been shown to increase the retention of ingested lead in experimental animals (*5*), probably by facilitating intestinal absorption of the metal. The question of the prophylactic efficacy of milk in lead poisoning in humans has been debated for years but not resolved (*6*).

One difficulty associated with analyzing the metabolic effects of milk is its chemical complexity. Hamilton (*7*) has argued, for example, that the facilitation of lead retention observed in rodents fed a milk diet (*5*) is an indirect result of the low iron content of milk, since iron deficiency has been shown repeatedly to increase the absorption and retention of ingested lead (*7, 8*). However, milk contains other materials that directly influence lead metabolism, including calcium, phosphorus, vitamin D, fat, and protein. Another constituent, unique to milk, is the milk sugar lactose, which also has profound effects on mineral metabolism in mammals. The absorption and retention of many minerals, including calcium (*9*), iron (*10*), zinc (*11*), manganese (*12*), cobalt (*13*), magnesium, strontium, barium, and rubidium (*14*), are enhanced by dietary lactose. To our knowledge, however, the effects of lactose on the absorption and retention of lead or other toxic metals have not been investigated. We report here that lactose, in physiological quantities, facilitates the intestinal absorption of orally administered lead in weanling rats and thus increases its uptake by tissues.

Male weanling rats (Holtzman Company, Madison, Wisconsin) were housed

Table 1. The effects of glucose or lactose on the absorption and tissue uptake of lead. Rats which were 26 days old and which had fasted for 24 hours were intubated with 1.0 ml of a dosing solution containing 0, 1, 3, or 6 mg of sugar per gram of body weight and 4.0 μCi of ^{210}Pb. The rats were killed 18 to 22 hours later. Tissues were assayed for radioactivity as described in the text. Values shown are the mean (± the standard error) percent of administered dose absorbed from the gastrointestinal tract (percentage absorption) or the mean percent of administered dose per gram of tissue (percentage uptake).

Treatment	Dose (mg/g)	*N*	Absorption (%)	Uptake by			
				Femur (%)	Kidneys (%)	Liver (%)	Blood (%)
Water (control)	0	10	38.7 ± 5.1	3.40 ± 0.59	3.89 ± 0.65	0.76 ± 0.13	0.52 ± 0.09
Glucose	1	12	40.8 ± 7.2	3.35 ± 0.62		0.84 ± 0.15	0.55 ± 0.10
Glucose	3	10	44.5 ± 2.7	4.13 ± 0.32	4.68 ± 0.41	0.85 ± 0.06	0.60 ± 0.05
Glucose	6	11	42.7 ± 3.6	2.83 ± 0.28	3.70 ± 0.24	0.77 ± 0.05	0.56 ± 0.04
Lactose	1	12	40.0 ± 5.2	3.35 ± 0.52		0.87 ± 0.14	0.61 ± 0.09
Lactose	3	9	74.7 ± 3.6*	6.74 ± 0.35*	7.31 ± 0.47*	1.52 ± 0.16*	0.90 ± 0.06*
Lactose	6	12	69.1 ± 3.0*	5.88 ± 0.33*	7.78 ± 0.91*	1.36 ± 0.10*	1.09 ± 0.08*

*Values that differ from the water control at $P < .01$.

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