

TABLE I

Effect of metabolic inhibitors on the excystment of cysts of *E. histolytica* induced by different agents

Inhibitors	Concentrations	Excystment agent							
		Live <i>E. coli</i>				L-amino acid mixture			
		I	IA	II	IIA	I	IA	II	IIA
Mytomycin C	1 µg/ml	Ex	15	Ex	43	Ex	21.4	Ex	50
Actinomycin D	1 µg/ml	Ex	25	Ex	43	Ex	22.2	Ex	33.3
Cycloheximide	2 × 10 <sup>-2</sup> M	Ex	17	Ex	16.5	Unex	Nil	Ex	10
Sod. Arsenite	10 <sup>-2</sup> M	Unex	Nil	Unex	Nil	Unex	Nil	Unex	Nil
Controls	—	Ex	87	Ex	87	Ex	80	Ex	80

Ex — Excysted. Unex — Unexcysted.

Expt I — Excystment in presence of excystment agent + inhibitors

Expt II — Excystment in presence of excystment agent after washing off the inhibitors

IA and IIA — Excystment percentage

L-amino acid mixture (isoleucine, arginine monohydrochloride, alanine, serine and glutamic acid)

excystment agent used (*E. coli* and L-amino acid mixture). In general the inhibition of excystment by cycloheximide and sodium arsenite shows that *de novo* protein synthesis and energy transduction mechanism of oxidative phosphorylation are essential, for both the activation of amoebae inside the dormant cysts, and their subsequent emergence from the cysts. Mitomycin C and actinomycin D have no apparent action on the activation of cysts or the emergence of amoebae.

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### ROLE OF NEUROTRANSMITTERS IN THE AESTIVATION OF THE INDIAN APPLE SNAIL, *PILA GLOBOSA* (SWAINSON)

P. MURALI MOHAN, K. YELLAMMA, K. SUBHASHINI AND K. SASIRA BABU  
Department of Zoology, Sri Venkateshwara University, Tirupati 517502, India.

EARLIER investigations<sup>1,2</sup>, on the nervous system of the aestivating snail, *Pila globosa*, revealed a decrease in the spontaneous activity and conduction velocities and an increase in the threshold values for different nerves. The decrease in electrical activity was correlated to the marked drop in the metabolism of the snail during aestivation. Murali Mohan and Babu<sup>3</sup> suggested an inhibitory role for glutamic acid during aestivation. As a followup, the present study attempts to elucidate the significance of the neurotransmitters acetylcholine (ACh) and glutamate during this torpid state in this snail.

The snails were aestivated by embedding them in sand in wooden boxes<sup>1</sup>. The ACh content<sup>4</sup>, acetylcholinesterase (AChE) activity<sup>4</sup>, and glutamic acid levels<sup>5</sup> in the central nervous system, consisting of all the ganglia, connectives and commissures, of normal and three months aestivated snails, were estimated by methods described earlier.

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The responses to electrical stimulation of the left pleurovisceral connective in normal and aestivated snails were recorded by the conventional electrophysiological set-up. The connective in normal and three months aestivated snails was exposed and washed with *Pila* Ringer<sup>6</sup>. After recording the control, the connective was perfused for 5 min with the test solutions ( $10^{-4}$  M ACh,  $10^{-3}$  M glutamic acid) prepared in Ringer which was again recorded.

TABLE I

Levels of ACh content, AChE activity and glutamic acid content in the nervous system of normal and three months aestivated *Pila globosa*

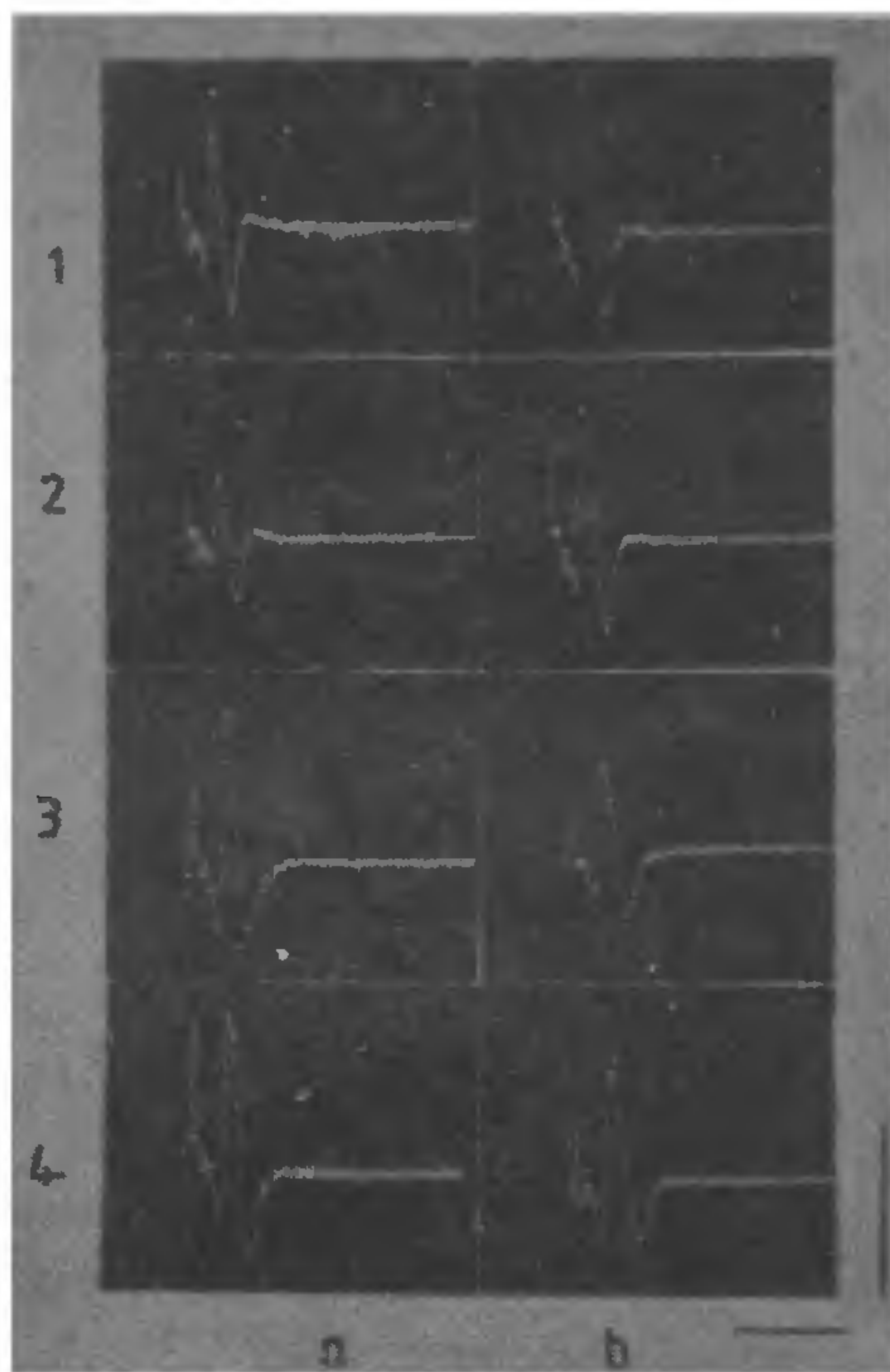
Parameter	Normal snails	Aestivated snails
ACh ( $\mu$ mol/g wet wt)	10.0 $\pm 1.0$	4.5 $\pm 0.3$
AChE ( $\mu$ mol/mg protein/h)	42.2 $\pm 3.8$	25.1 $\pm 2.1$
Glutamic acid ( $\mu$ mol/g wet wt)	2.5 $\pm 0.5$	12.5 $\pm 1.0$

Values are means of 6 individual observations  $\pm$  S.D. For each observation tissue from 3 to 4 animals was pooled. All values are significant at  $P < 0.001$ .

The results on biochemical estimations (table I) showed a decrease of 55% in the ACh content of the nervous system on aestivation. A corresponding decrease of 59% was also noticed in the associated AChE activity. Contrary to this, the glutamic acid content of the nervous system increased by 400% on aestivation.

Electrical recordings for stimulation of the left pleurovisceral connective showed that ACh has an elevatory effect on the electrical activity in both normal and aestivated snails (figures 1 and 2). On the contrary glutamic acid was found to inhibit the electrical activity (figures 3 and 4).

The role of ACh and glutamic acid as neurotransmitters in molluscan nervous systems has been explained earlier<sup>7</sup>. The present results suggest that different neurotransmitters could excite and inhibit the nervous system of *Pila*. Thus the snail, as a consequence of aestivation, where dormancy in all the segments of its physiology is implicated, adjusts the levels of different neurotransmitters to effect an increase in the levels of inhibitory transmitters and decrease in the levels of excitatory transmitters. This goes hand-in-hand with the changes in the electrical activity and general physiological state of the snail during aestivation.



Figures 1-4. Effect of ACh (1,2) and glutamic acid (3,4) on the electrical activity in normal (1,3) and aestivated (2,4) snails. (a) Control response, (b) Response on treatment with the test solution. Time 50 ms, Amplitude 200 mV.

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