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Hemicholinium-3: Noncholinergic Effects on Squid Axons

Abstract. *Hemicholinium-3, when applied to the inside of a squid axon, is effective in blocking the action potential. This action is not antagonized by the addition of choline or acetylcholine to the perfusate. Voltage-clamp experiments show that hemicholinium-3 depresses both the early transient and late steady-state components of membrane ionic conductances, with a greater effect on the peak transient component.*

Hemicholinium-3 (HC-3) inhibits effectively the synthesis of acetylcholine (*1*). This action of HC-3 has been attributed to a competitive inhibition of active choline transport across biological membranes (*2*). Hemicholinium-3 blocks impulse propagation in adrenergic (*3*) and frog sciatic (*4*) fibers. To decide whether HC-3 might have an effect on the sodium or potassium conductance change of nerve membranes which could explain its action on peripheral nerves without the necessity of invoking a cholinergic mechanism, we conducted experiments with single squid axons. We used standard microelectrode techniques in observations of membrane potentials and the sucrose-gap voltage clamp to measure ionic conductance changes.

Initially, we wanted to establish whether or not HC-3 had any effect on the resting and action potentials when applied either inside or outside the axon. Internal perfusion was carried out as follows (*5*). A giant axon was isolated and partially cleaned, its axoplasm was squeezed out by means of a small roller, and the crushed axon was inflated with an internal perfusate. All experiments with external applications were performed using isolated intact axon preparations. In all experiments the perfusion was continuous. The internal perfusate contained 400 mmole of K^+, 370 mmole of glutamate$^-$, 15 mmole of $H_2PO_4^-$, and 333 mmole of sucrose; the *p*H was adjusted to 7.3. Artificial seawater was used as the external bathing medium; it contained 449 mmole of Na^+, 10 mmole of K^+, 50 mmole of Ca^{2+}, 30 mmole of tris(hydroxymethyl)aminomethane, and 559 mmole of Cl^-, and the *p*H was adjusted to 8.0. A micropipette electrode was used to sense the resting and action potentials of the membrane. The maximum rate of rise of the action potential (*dv/dt*) was used as a measure of excitability because it is proportional to the inward current at that moment and is a more sensitive index than the height of the action potential. In those cases in which the resting potential slowly drifted, the measurements of the action potential and its rate of rise were made after the resting potential was brought back to its original level by application of an appropriate current through a separate microelectrode.

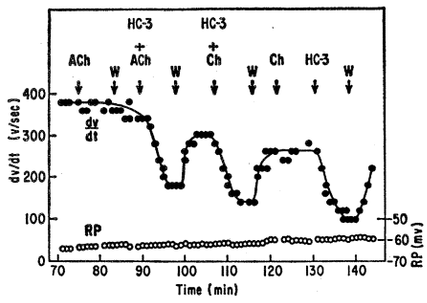

Fig. 1. Effect of internal applications of 1 mmole/liter of acetylcholine (ACh), choline (Ch), and hemicholinium-3 (HC-3) on the maximum rate of rise of the action potential (*dv/dt*) and the resting potential (RP); W refers to washing with normal internal perfusate.

Hemicholinium-3 had little or no effect on either the resting or action potential when applied externally in concentrations up to 10 m*M*. However, when 10 m*M* HC-3 was applied internally at this concentration the action potential was completely abolished with no obvious effect on the resting potential. Reducing the concentration to 1 m*M* still caused a significant depression of the rate of rise of the action potential. The effect was partially reversible with washing (*6*).

We repeated similar experiments with acetylcholine and choline chloride added to the perfusate to see whether these compounds exhibit any protective action against the blockage by internally applied HC-3 (Fig. 1). Choline (1 m*M*) or acetylcholine (1 m*M*) had no effect on the action or resting potential when given alone. When added to a 1 m*M* solution of HC-3 they did not inhibit the HC-3 action. These results indicate that the nerve blockage with HC-3 is not due to the lack of either choline or acetylcholine. However, the possibility that HC-3 is forming complexes with cholinergic structures in the membrane essential for

Table 1. Peak transient conductance (g_p) and late steady-state conductance (g_{ss}) before and after application of HC-3.

Preparation	Conc. (mmole/liter)	g_p (mmho/cm²)			g_{ss} (mmho/cm²)		
		Before	After	A/B	Before	After	A/B
			External application				
1	10	93	102	1.09	127	118	0.92
2	10	93	110	1.18	127	118	.92
3	10	91	83	0.91	105	92	.87
4	10	91	84	.92	105	86	.81
5	10	120	100	.83	108	83	.76
6	10	56	60	1.07	53	53	1.00
7	10	56	54	0.96	53	50	0.94
Mean				.99			.88
			Internal application				
1	10	96	27	0.28	76	62	0.81
2	10	96	23	.23	76	61	.80
3	10	100	30	.30	97	49	.50
4	10	114	33	.28	125	78	.62
5	10	129	43	.33	140	85	.60
Mean				.28			.66
6	1	113	79	0.69	125	89	0.71
7	1	101	71	.70	119	97	.81
8	1	101	74	.73	98	80	.81
Mean				.70			.77

impulse propagation cannot be ruled out.

In order to observe any HC-3–induced changes in membrane ionic conductance patterns, we used the sucrose-gap, voltage-clamp technique (*7*) (Table 1). As expected, when HC-3 was applied externally at a 10 m*M* concentration, there was very little effect on either the peak transient (sodium) or late steady-state (potassium) components of membrane conductance. The average value of the peak transient conductance following HC-3 is 99 percent of the control values with the late steady state being 88 percent. However, when applied internally, 10 m*M* HC-3 significantly reduced both the peak transient and late steady-state components. The greater effect was seen on the peak transient component which was reduced to an average value of only 28 percent of control as compared to 66 percent for the late steady-state component. Hemicholinium-3 was able to inhibit both conductance components at a lower internal concentration of 1 m*M*. Again, the effects were partially reversible with washing.

The action of HC-3 on squid axons can best be ascribed to an effect on the ionic conductance mechanisms of the nerve membrane, particularly the peak transient component rather than to a cholinergic mechanism.

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Vitamin A Deficiency: Effect on Mosquito Eye Ultrastructure

Abstract. *Mosquitoes* (Aedes aegypti) *were reared aseptically for one generation on an artificial diet containing neither vitamin A nor its usual precursor in animals, β-carotene. Function (electrical response to light) in the compound eyes of these animals was severely impaired. Ultrastructure of the photoreceptor cells was abnormal in two respects: multivesicular bodies were absent, and masses of smooth membrane lamellae were present near the nucleus. The organization of the photoreceptor organelle, the rhabdomere, was normal. The eyes of control mosquitoes, to whose diet β-carotene was added, were functionally and structurally normal. Multivesicular bodies were normally abundant and the perinuclear membrane masses were not present.*

In vitamin A deficiency, sensitivity of the vertebrate photoreceptor cell to light progressively decreases until blindness occurs and the photoreceptor organelle, the outer segment, degenerates (*1*). The biochemistry of vertebrate and insect vision is similar (*2*), and vitamin A deficiency results in decreased visual sensitivity in flies (*3*). A study with the light microscope revealed some morphological changes in the eye of a moth (*4*).

We have examined the ultrastructure of photoreceptor cells in the compound eyes of yellow fever mosquitoes (*Aedes aegypti* L.) with an induced vitamin A deficiency. Eye morphology (*5*) was compared in adult mosquitoes grown from hatching with or without a source of vitamin A in the larval diet. During the late larval period, the adult eye differentiates. The functional state of the photoreceptors was determined through measurement of electrical response to light (*6*). Three groups of mosquitoes were compared: (i) mosquitoes reared on the nonsterile, undefined diet (dry dog food crumbled in the water of the culture dishes) of the laboratory colony (group 1); (ii) mosquitoes reared aseptically on the minimum standard diet of Akov (*7*) with β-carotene added (0.077 mg/ml) as a vitamin A source (group 2); and (iii) mosquitoes reared aseptically on Akov's diet without a β-carotene supplement (group 3).

Electrical responses from the three groups were similar in wave form but differed in amplitude (Fig. 1). Mosquitoes reared on the defined diet without the β-carotene supplement (group 3) gave responses significantly smaller than those obtained from groups 1 and 2, whose eyes were functionally normal. The decrease of responsiveness in the vitamin A–deficient animals was probably due to a reduction in the amount of visual pigment present, for the wave form of the responses did not differ.

The fine structure of the normal mosquito compound eye is similar to that of other arthropod eyes (*8*). The ultrastructure of the receptor cells was identical in mosquitoes reared on dog food (group 1) and on the artificial diet with added β-carotene (group 2). By contrast, the eyes of those reared on the defined diet without β-carotene (group 3) were abnormal in the following respects. Multivesicular bodies (organelles characteristic of the photoreceptor cells of arthropods) were rarely found (compare Fig. 2, a and b), and masses of endomembrane were found at the proximal ends of the cells (Fig. 2, c and d). These masses, not normally present, were always found

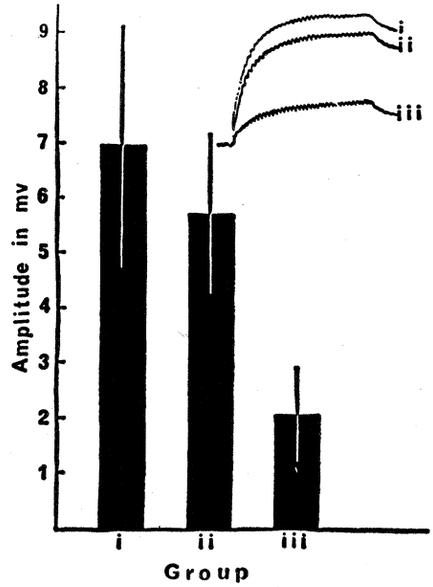

Fig. 1. Magnitude of the negative waves (taken just before cessation of the stimulus) of the electrical responses of mosquito eyes to light. Animals in groups 1 and 2 were reared on diets containing sources of vitamin A, those in group 3 on a diet lacking vitamin A. Each group contained 18 to 23 animals. The vertical lines represent the 95 percent confidence intervals. The inset shows typical electrical responses from each of the three groups.