# SHORT COMMUNICATION

# ACETYLCHOLINE DEPOLARIZES BARNACLE PHOTORECEPTORS

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A variety of vertebrate and invertebrate sensory cells are known to be sensitive to acetylcholine (ACh), although the purpose of ACh receptors on these cells is not understood. Acetylcholine elicits action potentials from the sensory cell of the crayfish stretch receptor organ, which receives inhibitory but not excitatory synaptic input (Wiersma, Furshpan & Florey, 1953). Among the vertebrates ACh causes spiking in several visceral and somatic sensory cells, including chemoreceptors and baroreceptors of the carotid body (von Euler, Liljestrand & Zotterman, 1941; Diamond, 1955), mechanoreceptors in the skin (Brown & Gray, 1948) and pain receptors (Skouby, 1951). The experiments to be described here demonstrate that ACh depolarizes the median photoreceptor of the giant barnacle, and test the possibility that the photoreceptor receives cholinergic synaptic input.

A preparation including the median ocellus, median ocellar nerve and the supraeosophageal ganglion, in which the photoreceptor axons terminate, was dissected from the giant barnacle, *Balanus nubilus*, and intracellular recordings were made from photoreceptor axons as described by Hudspeth & Stuart (1977). Acetylcholine and other compounds were applied to the preparation by perfusion of the experimental chamber. Normal physiological saline contained, in $mmol\,l^{-1}$: NaCl, 462; KCl, 8; $CaCl_2$, 20; $MgCl_2$, 12; and Tris-HC1, 10; adjusted to pH 7·7 (Brown, Hagiwara, Koike & Meech, 1970).

Acetylcholine reversibly depolarizes the membrane of the photoreceptor (Fig. 1). Most photoreceptors tested were depolarized 1–3 mV by $10^{-8}\,mol\,l^{-1}$ ACh. Two photoreceptors, not included in the dose-response curve of Fig. 1, were particularly sensitive, responding to $10^{-8}\,mol\,l^{-1}$ ACh with 8 mV and 14 mV depolarizations. The sensitivity of the photoreceptor to ACh is therefore comparable to that of cells known to receive cholinergic synaptic input.

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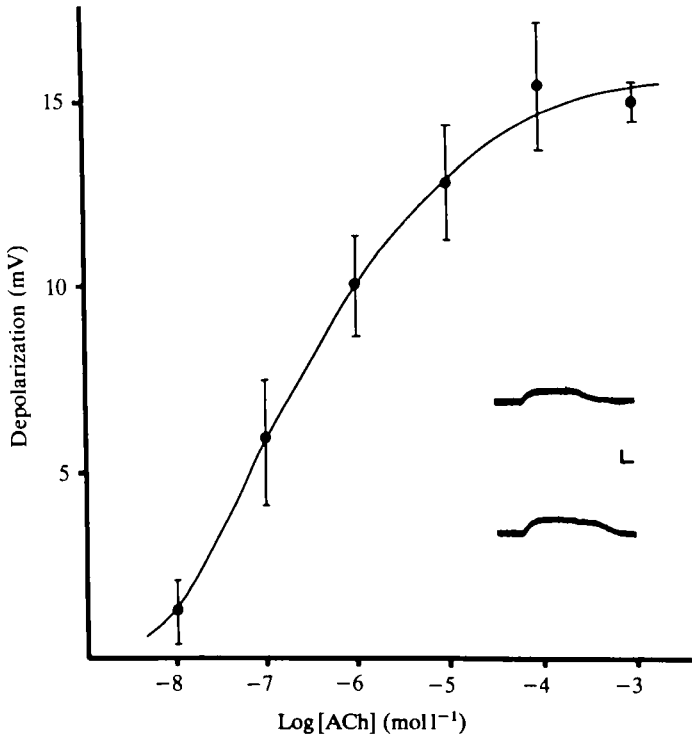Key words: Acetylcholine, barnacle, photoreceptor.

Fig. 1. Acetylcholine depolarizes the membrane of the photoreceptor. The points of the dose-response curve are averages from eight or more experiments, with the exception of the response to $10^{-3}$ mol l$^{-1}$ ACh, which is the average from three experiments. The error bars measure standard deviations. Inset: the *upper trace* shows a depolarizing response to $10^{-6}$ mol l$^{-1}$ ACh. The membrane potential returned to its resting value when the ACh was washed from the bath after about 5 min. In the *lower trace* the same photoreceptor was exposed to $10^{-6}$ mol l$^{-1}$ ACh in saline containing 22 mmol l$^{-1}$ Co$^{2+}$ and 10 mmol l$^{-1}$ Ca$^{2+}$. The reason for the slightly greater depolarization in 22 mmol l$^{-1}$ Co$^{2+}$ saline is not known. Normal saline contains 20 mmol l$^{-1}$ Ca$^{2+}$ and 12 mmol l$^{-1}$ Mg$^{2+}$. Calibration: 5 mV, 1 min.

Since neurones of the ganglion in which the photoreceptor axons terminate were exposed to superfused ACh, it seemed possible that ACh was depolarizing the membrane of some neurone other than the photoreceptor, which might in turn depolarize the photoreceptor through an excitatory synapse. To test this possibility the preparation was exposed to ACh in a saline containing 22 mmol l$^{-1}$ Co$^{2+}$ and lowered (10 mmol l$^{-1}$) Ca$^{2+}$. This saline blocks transmission from the photoreceptor to the cell directly postsynaptic to it (L. C. Timpe, unpublished observation). The inset to Fig. 1 shows that the photoreceptor's response to $10^{-6}$ mol l$^{-1}$ ACh is undiminished in this saline. Therefore ACh acts directly on the membrane of the photoreceptor.

To determine whether ACh causes a conductance change in the photoreceptor, the membrane potential was manually 'clamped' at the dark resting level during ACh application. As $10^{-6}$ mol l$^{-1}$ ACh was perfused through the bath, the depolarization which would normally occur was offset by hand-controlled hyperpolarizing current injected through a second microelectrode (Fig. 2A). Acetylcholine ($10^{-6}$ mol l$^{-1}$) reduced the voltage change in response to constant, periodic current injections, from which we conclude that it reduced the photoreceptor's input resistance.

Which ion carries the ACh-induced current? Saline containing $22\,\mathrm{mmol\,l^{-1}}$ $Co^{2+}$ and $10\,\mathrm{mmol\,l^{-1}}$ $Ca^{2+}$ did not reduce the depolarization caused by ACh, suggesting that $Ca^{2+}$ is not the primary ion carrying the current (Fig. 1, inset). When NaCl was replaced by sucrose, but divalent cations were kept normal ($20\,\mathrm{mmol\,l^{-1}}$ $Ca^{2+}$, $12\,\mathrm{mmol\,l^{-1}}$ $Mg^{2+}$), ACh was unable to depolarize the photoreceptor. Thus sodium ions carry a large part of the depolarizing current.

The nicotinic antagonists dihydro-$\beta$-erythroidine (d-$\beta$-e), and d-tubocurarine reduced the amplitudes of ACh-induced depolarizations in the photoreceptor. At $10^{-6}\,\mathrm{mol\,l^{-1}}$, d-$\beta$-e reduced by about half the depolarization caused by $10^{-6}\,\mathrm{mol\,l^{-1}}$ ACh (Fig. 2B). Dihydro-$\beta$-erythroidine ($10^{-6}\,\mathrm{mol\,l^{-1}}$) also blocked the increase in membrane conductance caused by ACh. Curare is a less potent antagonist of ACh than d-$\beta$-e, requiring a concentration of approximately $10^{-4}\,\mathrm{mol\,l^{-1}}$ to reduce by half the depolarizing effect of $10^{-6}\,\mathrm{mol\,l^{-1}}$ ACh. In one experiment, $2 \times 10^{-6}\,\mathrm{mol\,l^{-1}}$ $\alpha$-bungarotoxin reduced the depolarization caused by $10^{-6}\,\mathrm{mol\,l^{-1}}$ ACh by about half. This antagonism reversed, though slowly (2h). In two experiments nicotine depolarized the membrane of the photoreceptor, although it was about 100 times less effective than ACh.
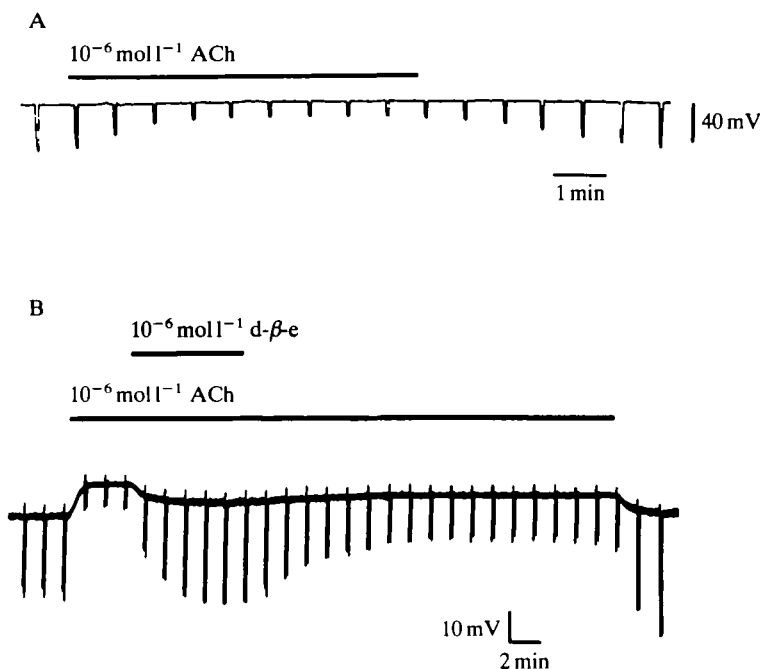


Fig. 2. Acetylcholine increases the conductance of the photoreceptor's membrane. (A) As $10^{-6}$ $\mathrm{mol\,l^{-1}}$ ACh entered the bath, hyperpolarizing current was injected to counterbalance the ACh-induced depolarization. Hyperpolarizing current pulses (1 nA) at 45-s intervals tested the input resistance. $10^{-6}\,\mathrm{mol\,l^{-1}}$ ACh reduced the steady-state input resistance. The experiment was done with two intracellular electrodes, one for injecting current and the other for recording membrane potential. (B) Dihydro-$\beta$-erythroidine (d-$\beta$-e) antagonizes the ACh-induced depolarization. $10^{-6}\,\mathrm{mol\,l^{-1}}$ d-$\beta$-e reduced by about half the depolarization caused by $10^{-6}\,\mathrm{mol\,l^{-1}}$ ACh, and also blocked the ACh-induced conductance increase. The effect of d-$\beta$-e reversed only after prolonged washing. Hyperpolarizing current pulses were applied across a bridge circuit. As the experiment progressed the tip resistance of the microelectrode increased and the bridge became unbalanced.

The anticholinesterase eserine was applied to the preparation along with ACh to see if it would enhance the photoreceptor's response to ACh, thereby suggesting the presence of the enzyme near the ACh receptors. Eserine ($10^{-6}$ mol l$^{-1}$), however, reduced rather than increased the photoreceptor's response to $10^{-6}$ mol l$^{-1}$ ACh. When applied by itself, at concentrations of $10^{-6}$ mol l$^{-1}$ and above, eserine depolarized the photoreceptor. These observations suggest that eserine is a partial agonist of the photoreceptor's ACh receptor.

The photoreceptor's sensitivity to ACh suggested that it may receive cholinergic synaptic input. We sought evidence for such an input by stimulating extracellularly each nerve entering the supraoesophageal ganglion, including the antennular, supra-splanchnic and circumoesophageal connective nerves, plus a small nerve which leaves the ganglionic commissure and innervates the oesophagus (Gwilliam & Cole, 1979). We were unable to elicit synaptic activity in the photoreceptor.

If the presynaptic element were a non-spiking neurone, stimulating its axon might not depolarize the presynaptic endings enough to release neurotransmitter. The experiments were repeated in 5 mmol l$^{-1}$ tetraethylammonium ion, which allows stimulated median photoreceptors to support $Ca^{2+}$ spikes (Ross & Stuart, 1978), and might also permit spikes to be set up in other, unidentified, decrementally conducting neurones. Excitation of the various nerves still produced no synaptic potentials in the photoreceptor. Since the antennular nerves contain the axons of the lateral photoreceptors, this experiment (in addition to experiments by Ms L. Oland, in preparation) suggests that lateral photoreceptors do not synapse onto median photoreceptors.

We tested the possibility that ACh is released from a higher order visual cell back onto the photoreceptor. Klingman & Chappell (1978) have presented evidence for such a cholinergic feedback synapse in the dragonfly, where the responses of the photoreceptor and the second order cell are similar to those of the barnacle. To test whether interference with the reception of ACh would affect the response to light in the barnacle's photoreceptor (as it does in a dragonfly), the preparation was superfused with d-$\beta$-e or curare. Neither the peak, the plateau nor the hyperpolarizing undershoot of the light response was changed in $10^{-5}$ mol l$^{-1}$ d-$\beta$-e, a concentration sufficient to block completely the effect of superfused $10^{-6}$ mol l$^{-1}$ ACh. In five experiments there was no change in the photoreceptor's response to light of any intensity.

Experiments were done to determine whether the ACh receptors are confined to the photoreceptor's arborizations in the neuropile or whether they are more widely distributed on the photoreceptor. An experimental bath with two chambers was used to isolate the saline superfusing the ocellus and axon from that superfusing the photoreceptor's presynaptic terminals (Ozawa, Hagiwara, Nicolaysen & Stuart, 1975). Perfusing ACh through either chamber depolarized the photoreceptor's membrane, even when the other chamber contained a high concentration of curare. In 10 experiments of this type, in which the position of the barrier between chambers was varied along the length of the median ocellar nerve, ACh in either chamber was able to depolarize the photoreceptor's membrane. Thus sensitivity to ACh is widespread in the photoreceptor.

What is the function of this ACh receptor, if it does not subserve synaptic input? It has been suggested that the release and reception of ACh might play a role in

sensory transduction (Davis, 1961) or in the propagation of the light response along the photoreceptor's axon (Nachmanson & Neumann, 1975). In this photoreceptor the ACh receptors are not participating in either transduction or propagation: blocking the ACh receptor with $10^{-5}$ mol l$^{-1}$ d-$\beta$-e does not change the shape of the normal response to light, nor does it interfere with the spread of this response from the ocellus down the axon to the terminals.

Another possibility is that ACh has a hormonal action (Florey & Cahill, 1980) on the photoreceptor, perhaps controlling its resting membrane potential. In this connection it would be of interest to examine the barnacle's haemolymph for the presence of ACh.

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