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SHORT COMMUNICATION

SYNAPTIC CHLORIDE CHANNELS GENERATING HYPERPOLARIZING ON-RESPONSES IN MONOPOLAR NEURONES OF THE BLOWFLY VISUAL SYSTEM

BY F. ZETTLER AND H. STRAKA

Zoologisches Institut der Universität München, Luisenstrasse 14, 8000 München 2, FRG

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The large monopolar neurones L1 and L2 in the first optic ganglion of dipteran flies are postsynaptic to short-fibre photoreceptors (Trujillo-Cenòz, 1965; Boschek, 1971; Burkhardt & Braitenberg, 1976). Each neurone is contacted by six photoreceptor axons resulting in 1200 synaptic terminations (Nicol & Meinertzhagen, 1982). The photoreceptor response to light is a graded depolarization which, by synaptic transmission, is converted with considerable gain to a postsynaptic hyperpolarization (Järvilehto & Zettler, 1971). When presynaptic photoreceptors are stimulated with light of constant intensity, the postsynaptic potential (PSP) typically consists of a fast initial hyperpolarization (on-response) followed by a slower decay to a constant level about 100 ms after stimulus onset. Termination of the light stimulus results in an off-transient depolarization (the complete, normal PSP shape is shown in Fig. 1). All phases of the PSP are intensity-dependent. The present report is concerned with the hyperpolarizing on-response in the blowfly monopolar neurones.

It has been reported that membrane resistance in locust monopolar cells decreases during the hyperpolarization response of the PSP (Shaw, 1968). Similarly, a decrease in membrane resistance in dragonfly monopolar cells, combined with an on-response reversal potential that is 65 mV below the resting potential, has been interpreted as an increase in potassium and/or chloride conductance generating the on-response (Laughlin, 1974). Attempts to determine the nature of the ionic source generating the hyperpolarizing response of monopolar cells have been rather inconclusive. Intracellular injection of the potassium channel blocker tetraethylammonium (TEA^+) reduced the on-response in monopolar cells, implying that the normal on-response is generated by the opening of potassium channels (Zimmerman, 1978). However, these responses are open to an alternative interpretation, since application of TEA^+ also causes hyperpolarization of the resting potential. It has been shown that the reversal potential of the on-response is well below the resting potential (Zimmerman, 1978). As application of TEA^+ shifts the membrane potential towards the reversal potential, a reduction of the on-response would be expected, and it is

Key words: fly visual system, monopolar neurones, synaptic chloride channels, ion injection.

therefore unnecessary to implicate potassium channels in the generation of the hyperpolarizing on-response.

In the present study direct ionophoresis was used to change the intracellular ion composition. Isolated head preparations of the wild blowfly *Calliphora erythrocephala* were used to make intracellular recordings from monopolar neurones in the lamina region. Conventional electrophysiological recording techniques were used, including a bridge balance and current-clamping equipment for ionophoresis. The light stimulus consisted of a quartz–halogen bulb (100 W) focused at one end of a flexible light guide (3 mm in diameter), with the other end of the light guide illuminating the head preparation from a distance of 60 mm and positioned on-axis for the penetrated monopolar cell. A shutter and neutral filters were positioned between the bulb and the light guide. Microelectrodes were filled with 2 mol l^{-1} potassium acetate unless otherwise stated. Tip resistances were 100–120 MΩ.

Impalements of eight monopolar cells in the lamina region produced resting potentials of $-37 \pm 2{\cdot}3$ mV (±S.D.) and input resistances of $13 \pm 3{\cdot}9$ MΩ, based on the difference between extracellular and intracellular conditions. Membrane resistance changes were measured using ±0·5 nA current pulses of 15 ms duration. In these experiments electrode plus input resistance was compensated for by balancing the bridge under dark conditions. A decrease in the input resistance to 40 % of the value in the dark was associated with the fast on-transient response, recovering to 70 % of the dark value 100 ms after the initial response. From an average of six impaled cells, the reversal potential was found to be 55 mV below the resting potential, as determined from a series of pre-hyperpolarizations; in this case, electrode resistance was balanced extracellularly to zero.

Increasing the intracellular chloride content by electrophoretic injection considerably reduced the hyperpolarizing on-response. In a few cells polarity changes could be seen immediately after injection (Fig. 1A), with the resting potential remaining unaltered. Chloride ions were injected into six different cells from electrodes filled with either 1 mol l^{-1} choline chloride or 2 mol l^{-1} potassium chloride and the same effect was observed in each cell. For most of the impaled cells it was possible to repeat chloride injections after normal cellular conditions had been restored. The quantity of injected chloride ions was varied from 10 to 100 nC and reduction of the on-response was correlated to the charge injected (Fig. 1B). To exclude possible non-specific current effects, five impaled cells were tested by injection of acetate ions instead of chloride ions. No changes in PSP response or resting potential were observed following ion injection (Fig. 2A)

There are no data on intracellular ionic composition in monopolar neurones, but it is generally agreed that intracellular chloride concentration in many cells may be considerably lower than extracellular concentration. Thus, it is reasonable to suggest that the equilibrium potential for chloride ions is negative to the resting potential (−37 mV). Increasing intracellular chloride concentration by injection can be expected to shift the equilibrium potential towards and possibly beyond the resting potential. On this basis, the effects demonstrated in Fig. 1 can be interpreted most

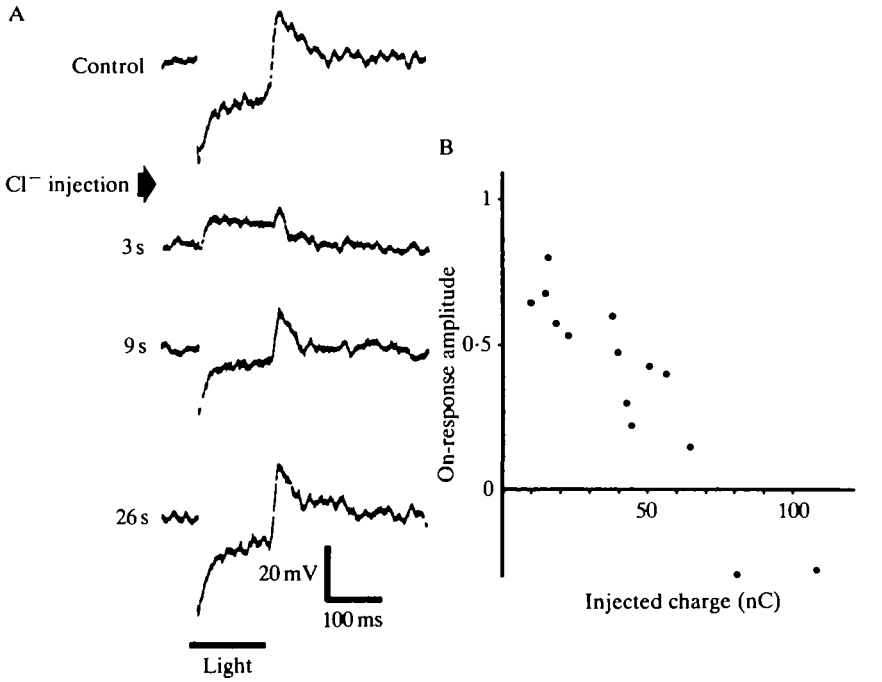

Fig. 1. Reduction of the on-response by intracellular injection of chloride ions. (A) Upper trace shows normal PSP wave form, elicited by a constant light stimulus of moderate intensity. Remaining traces illustrate changes in PSP response at different times after the cessation of an 8 s, 10 nA chloride injection. (B) Dependence of the on-response amplitude (from six cell impalements) on the amount of charge injected (nC). The on-response amplitude is measured immediately after an injection and plotted in arbitrary units normalized to the pre-injection value.

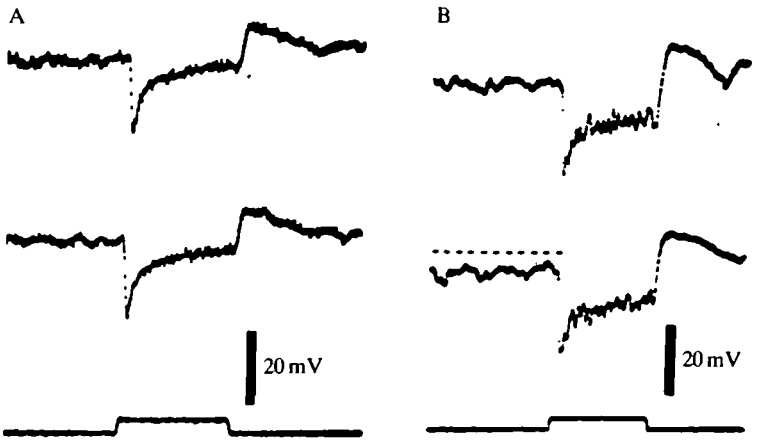

Fig. 2. (A) PSP response before (upper trace) and after (lower trace) acetate injection with a constant current pulse of −5 nA lasting for 17·5 s. (B) PSP response from another cell before and after injection of potassium ions with a current pulse of 6 nA for 11 s. Dashed line indicates resting potential under normal conditions. Bottom traces in A and B represent the time trace of a 150 ms light stimulus of moderate intensity.

easily by assuming that an increase in chloride conductance is responsible for generating the on-response.

However, it is conceivable that both chloride and potassium channels may be involved in generating an on-response. To resolve this question, the intracellular potassium ion concentration was increased by ionophoretic injection in an attempt to lower the potassium equilibrium potential and, if potassium channels are involved, to increase the amplitude of the on-response. However, the effects of intracellular injection of potassium ions cannot be expected to be as potent as chloride ion injection because intracellular potassium concentration is normally much higher than chloride concentration. Thus, injection of equal amounts of potassium ions (compared to chloride) will change the intracellular concentration only slightly. Potassium ions are also assumed to contribute substantially to the resting potential, which should also be changed by increasing the intracellular potassium concentration.

Injection of potassium ions from electrodes filled with $2\,\text{mol}\,l^{-1}$ potassium acetate or $2\,\text{mol}\,l^{-1}$ potassium chloride resulted in a slight, but consistent, effect on the on-response and resting potential (Fig. 2B). The resting potential was lowered by potassium injection, as expected, but the amplitude of the on-response also decreased. Such an effect was small but occurred in all five monopolar cells tested. In four of these cells, ion injection was repeated several times, producing 12 standard injections of charge (100 nC). In these standardized injections the resting potential was lowered by $5{\cdot}8 \pm 2{\cdot}2\,\text{mV}$ and the on-response was reduced by $3{\cdot}6 \pm 2{\cdot}4\,\text{mV}$. These results suggest that potassium channels are unlikely to be involved in generating the hyperpolarizing on-response.

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