THE ACTION OF GABA AND SEROTONIN ON THE RESPONSE PROPERTIES OF RETINAL HORIZONTAL CELLS OF THE SKATE RAJA OSCELLATA

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The retina of the skate (Raja Oscellata) has been shown to possess neurons that contain the putative neurotransmitters GABA and serotonin (Brunken et al., J. Comp. Neur. 243: 1-12, 1986). GABA was found in an interplexiform type cell, while serotonin was located within amacrine cells. In teleost fish, interplexiform cells contain the neurotransmitter dopamine (Ehinger, Retina 2: 305-321. 1982) which has been shown to uncouple adjacent horizontal cells at their gap junctions, thus changing the receptive field properties of the cells (Teranishi et al., J. Neurosci. 4: 1271-1280, 1984). We therefore wanted to determine if the GABAergic interplexiform cell of the skate works the same way as the dopaminergic interplexiform cell found in teleost retinas. In addition we examined the effect of serotonin on the responses of horizontal cells to determine if it played a possble role in the activation of GABAergic interplexiform cells, since it has been found in amacrine cells of skates which may be presynaptic to the GABAergic interplexiform cells (Brunken et al., J. Comp. Neur. 243: 1-12).

Skates were dark adapted 2-3 hours before an experiment. Animals were pithed and an eye enucleated. The anterior section was removed, including cornea, lens and vitreous. A piece of the remaining eyecup was trimmed to approximately 1 cm^2, placed inside a perfusion chamber and held in place by a retaining ring. Elasmobranch saline flowed over the retina at a rate of 1-2 ml/min.

Light stimuli was delivered to the retina by means of a dual beam photostimulator. This provided two independent channels of light in which wavelength, intensity, size and position of the stimulus could be varied.

Standard intracellular recording procedures were used. Micropipettes were pulled on a Brown-Flaming puller and filled with 3M potassium chloride. These electrodes had resistance of 100-300 megohms when measured on the retinal surface. Electrodes were moved through the retina by means of a hydraulic microdrive.

The saline solution (250mM NaCl, 6mM KCl, 20 mM $NaHCO_3$, 1mM $MgCl_2$, 4mM $CaCl_2.2H_2O$, 0.2mM $NaH_2PO_4.H_2O$, 360 mM urea, 5 mM HEPES, 10 mM glucose) was continuously bubbled with 95% oxygen, 5% carbon dioxide and adjusted to pH 7.8 with NaOH before the start of the experiment. All drugs were dissolved in saline with the exception of cobalt chloride which was first made up as a stock solution in distilled water and then diluted with saline. The elasmobranch saline and test solutions were changed using a 6-way teflon valve.

Horizontal cells were identified by their characteristic response to specific types of light stimuli. Horizontal cells were encountered between 75 and 120 microns below the retinal surface. They had resting membrane potentials in the dark between -20mv and -40mv. These cells responded to light with graded hyperpolarizations that increased with increasing stimulus diameter.

Application of 500 uM GABA to the dark adapted retina and

stimulating with a large (> 1 cm) spot of light, caused a depolarization of the membrane potential of 7-12 mv, and an increase in the amplitude of the hyperpolarizing response (Fig. 1.). Retinas were then light adapted with background illumination 3 log units above threshold. When 500 uM GABA was then applied to the retina there was only a slight depolarization of the membrane potential 3-5 mv, with no increase in the amplitude of the hyperpolarizing response to light stimuli.

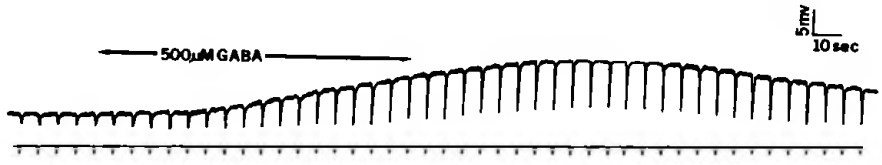

Figure 1. The effect of 500 uM GABA on the responses of a dark adapted horizontal cell in the retina of the skate. Gaba depolarized the membrane potential 11 mv and increased the amplitude to light-evoked stimuli. The retina was illuminated with 200 msec flashes of 500 nm light presented every 7 seconds.

To determine if GABA caused changes in the receptive field size, horizontal cells were impaled with microelectrodes and responses were recorded to stimuli consisting of a small spot of light (500 nm, 320 microns diameter) followed by an annulus (540nm, 1000 microns O.D., 400 microns I.D.) In a typical experiment, when 500 uM GABA was applied to the retina, the membrane potential depolarized and the response amplitude to the spot stimuli remained the same or decreased slightly, while the response to the annulus more than doubled in size from 5.2 mv to 11.2 mv. This increase in size of the response to annular stimulation implies that GABA causes a change in the receptive field profile of the horizontal ell. However, unlike the dopaminergic interplexiform cell of teleost fish which increases the response to spot stimulation and decreases the response to annular stimulation (Teranishi et al. J. Neurosci. 4: 1271-1280, 1984), the GABAergic interplexiform cell of the skate appears to work in an opposite manner, i.e. it increases

the response to large stimuli. It is unclear yet whether the change in receptive field profile of the skate horizontal cells upon application of GABA works by the same mechanism as that found in teleosts.

In another series of experiments, the effect of 500 uM serotonin was observed on the response properties of skate horizontal cells. When serotonin was applied to the superfusate, the membrane potential of the horizontal cells depolarized approximately 13 mv. The response to large spots of light was an increase in their amplitude. However, there was no differential increase in the amplitude between a spot and annulus of light when serotonin was applied to the retina.

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