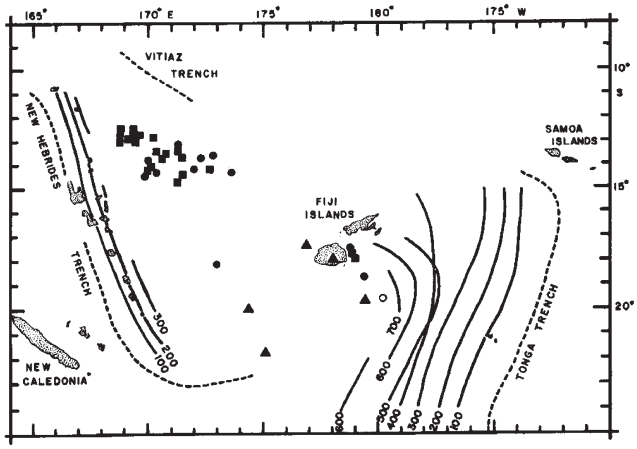

Fig. 5 Map showing contours of earthquake depths for Tonga and New Hebrides arcs, the deep seismic zone of New Hebrides, and well located deep earthquakes beneath the Fiji islands and the Fiji plateau. ▲, Events with depth range between 525 and 575 km; ●, events with 576 to 625 km depth; ■, events with 626 to 675 km depth. The open circle represents an event with a depth of 470 km.

of the inclined seismic zone of the Tonga arc (Fig. 5). In the past 10 yr about 8 well located deep earthquakes occurred beneath the Fiji Islands. It is not clear whether these earthquakes represent a continuation of the descending Tonga slab, a slab(s) detached from the present descending slab, or a slab detached during an earlier episode of underthrusting. Evidence obtained from focal mechanisms by Isacks *et al.*[14] suggests that these earthquakes represent a contorted continuation of the northern edge of the descending Tonga slab. In any case these earthquakes and the New Hebrides deep earthquakes show a considerable horizontal extent away from the corresponding descending slabs. This suggests that the earthquakes mark lithospheric slabs that are unable to penetrate the 600–700 km discontinuity of the upper mantle, and hence the discontinuity may represent the lower limit of the asthenosphere. These slabs may pile up above the discontinuity until their assimilation in the mesospheric lower mantle.

Overall Model

The New Hebrides and New Zealand deep earthquakes mark detached pieces of lithosphere in the upper mantle; considerable seismic wave attenuation probably exists below about 300 km of depth in the upper mantle beneath the northern part of the New Hebrides arc, which implies that the asthenosphere may extend deeper than 300 km in the mantle in this region; the spatial distribution of deep earthquakes between the New Hebrides and Tonga arcs suggests that slabs of lithosphere are unable to descend beneath about 700 km in the mantle.

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Effect of Magnesium on Horizontal Cell Activity in the Skate Retina

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Magnesium, like light, hyperpolarizes retinal horizontal cells. In the dark, horizontal cells seem to be depolarized by neurotransmitter released continuously from photoreceptors.

THERE is both anatomical and physiological evidence to suggest that synaptic transmission between vertebrate photoreceptors and second-order retinal neurones (horizontal and bipolar cells) is chemically mediated. The aggregation of vesicles in the receptor terminals[1], the separation between junctional membranes[2,3], and the latency differences between pre- and postsynaptic responses[4] are consistent with this view. Although identification of the neurotransmitter has yet to be made, studies on the properties of horizontal cells have led to the interesting suggestion that the photoreceptor releases a depolarizing transmitter in the dark and that light decreases the flow of this substance[5–8]. For example, horizontal cells exhibit low resting potentials (25–40 mV)[9,10], and most of

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these cells only hyperpolarize in response to light[9]. Furthermore, an increase of input resistance of the horizontal cell often accompanies light stimulation[11], and transretinal currents that depolarize the receptor terminals induce a depolarizing response in the horizontal cells[5–8]. Recent studies on vertebrate photoreceptors have also provided results compatible with this notion. In darkness there is a steady inward sodium flux across the plasma membrane of the rod outer segment; light decreases the sodium conductance of the outer segment, thereby hyperpolarizing the receptor[8,11,12]. Thus, the receptors are partially depolarized in the dark, a finding consistent with the suggestion of neurotransmitter release in darkness.

We have tested this hypothesis by determining the influence of magnesium on the electrical properties of skate horizontal cells (either *Raja erinacea* or *R. oscellata*). We assumed that, as at other chemically-mediated synapses, high levels of extracellular magnesium block neurotransmitter release from the presynaptic (that is receptor) terminal[13–15]. Intracellular recordings from horizontal cells were obtained from the eyecup preparation with glass micropipettes filled with 2 M KCl and having resistances of 35–55 Mohm when measured extracellularly in the tissue[10]. Signals were led from the micropipettes to a Bak ELSA-4 negative-capacitance amplifier via Ag-AgCl electrodes, observed on an oscilloscope, and recorded on a Brush (model 280) penwriter. In addition, recordings of the electroretinogram (ERG) were obtained with a partially insulated Ag-AgCl wire placed in the vitreous humour. ERG responses were amplified, displayed and recorded conventionally[16,17]. After impaling a horizontal cell, a drop of elasmobranch Ringer[18], in which 100 mM MgCl was substituted for 100 mM of NaCl, was gently pressure-ejected onto the surface of the retina. Recordings were obtained before, during, and after exposure to the high magnesium Ringer.

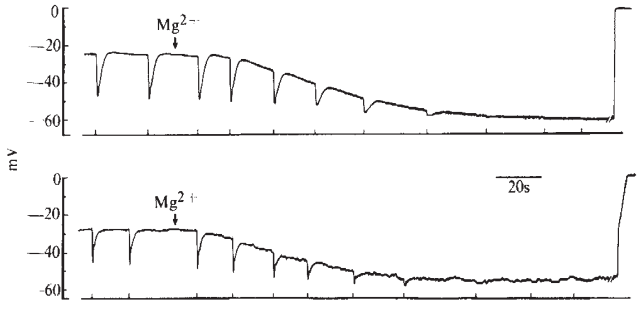

Fig. 1 Two experiments showing the effects of magnesium on skate horizontal cells. A drop of Mg-Ringer was applied to the eyecup (arrows), and within 15–25 s the cells began to hyperpolarize. Over the next 3 min, the cells hyperpolarized to a level of about −60 mV and light evoked activity was lost. A break in the trace indicates the time at which the pipettes were withdrawn from the cells. The rapid, positive shifts of potential of 55 to 60 mV confirmed the increase in membrane potential of the horizontal cells in the presence of high levels of magnesium. Flash intensity (filter density of 5.5) and duration (0.2 s) were kept constant throughout both experiments. The markers along the lower trace of each record indicate flash preparations.

Fig. 1 shows the effects of the test solution (Mg-Ringer) on two horizontal cells. Resting potentials of 25 and 30 mV were recorded initially in the two cells and light flashes of constant duration and intensity gave hyperpolarizing responses of 15–20 mV. The test stimuli were about 1.5 log units above threshold; flashes of saturating intensity evoked responses of 25–30 mV in these cells.

About 15–25 s after a drop of Mg-Ringer was applied to the eyecup (arrows, Fig. 1), the membrane potentials of the horizontal cells began to hyperpolarize and there was a corrresponding decrease in the amplitudes of the light-evoked responses. Within 3 min, the membrane potentials had fallen to about −60 mV and the response to light was abolished. In these two experiments, the micropipettes were withdrawn from the cells after the resting potentials had stabilized. Positive shifts of potential of 55–60 mV signalled the exit of the pipettes from the cells and confirmed the intracellular measurements of large membrane potentials in the presence of high magnesium.

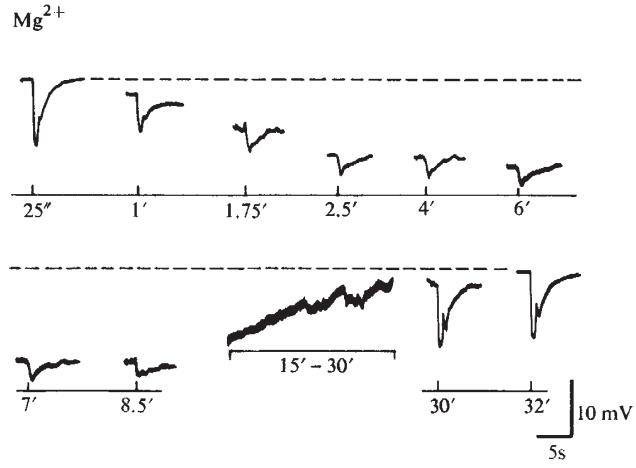

Fig. 2 Long-term recordings illustrating the transient nature of the effects of magnesium on skate horizontal cells. The times after the application of the test solution at which light stimuli were presented are indicated along the lower trace. The level of each trace relative to the dashed line shows the extent to which the cell was hyperpolarized. The cell was not completely hyperpolarized by the drop of test solution, so a small response could be elicited even after 8.5 min. Between 15 and 30 min (shown on the contracted time base) the membrane potential of the cell depolarized, and the responses at the end of this time had returned to normal amplitudes.

These results strongly support the notion that skate horizontal cells are maintained in a partially depolarized state in the dark, because of continual release of a depolarizing neurotransmitter from the photoreceptors. On this view, high magnesium, like light, decreases the release of neurotransmitter and the horizontal cell hyperpolarizes. As the horizontal cells hyperpolarize in response to a high magnesium environment, there is a distinct increase in baseline fluctuations. This is particularly evident in the lower record of Fig. 1. It is possible that these fluctuations indicate the presence of spontaneous miniature postsynaptic potentials in the horizontal cells, which are uncovered in conditions of high extracellular magnesium. In muscle it has been shown that miniature endplate potentials persist in the presence of high magnesium[19,20].

The effects of a drop of Mg-Ringer on the eyecup preparation are transient. Fig. 2 illustrates an experiment in which a horizontal cell was monitored for more than 30 min after the test solution was added to the eyecup. In this experiment the horizontal cell did not fully hyperpolarize and light-evoked responses were not entirely abolished. After application of the Mg-Ringer for 30 s, the membrane potential of the cell became more negative and responses to light decreased in amplitude.

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After 3–5 min the resting potential stabilized, and only small light-evoked potentials could be recorded. For the next 5–10 min, no significant changes were noted. Between 15 and 30 min, however, the horizontal cell membrane depolarized to its former level (~ -30 mV) and light-evoked activity regained its original amplitude.

Earlier experiments by Svaetichin and his colleagues[21,22] showed that anoxia and a variety of metabolic poisons produce effects on horizontal cells similar to those reported here. Some of these agents may exert their effects by disrupting synaptic transmission, but others may inactivate the receptors as well. The ERG was not monitored in those experiments; no information on receptor activity in the presence of the agents was provided.

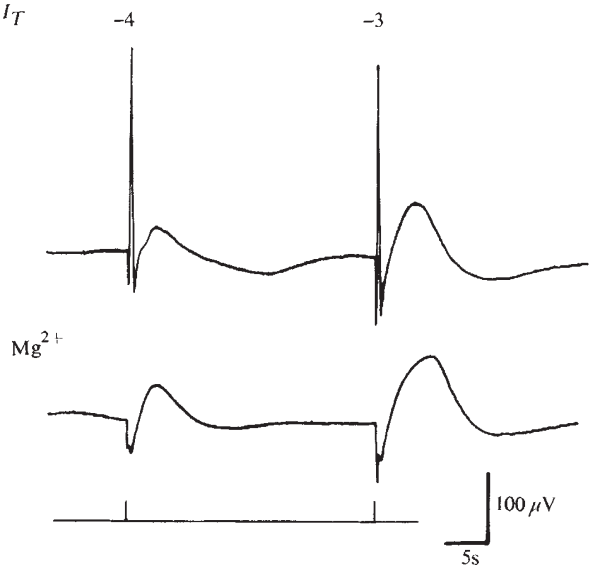

Fig. 3 The effects of Mg-Ringer on the skate electroretinogram (ERG). The upper traces show the principal components of the response at two flash intensities (filter densities of 3 and 4) in the normal ERG. The initial negative deflexion (a-wave) is followed by the large positive b-wave and a slow c-wave of lower amplitude. Magnesium almost entirely abolished the b-wave, but a- and c-waves are virtually unaltered.

Fig. 3 shows ERG recordings at two intensities ($I_t = -3$ and -4) before and 15–20 min after the Mg-Ringer was applied to the eyecup. The a- and c-waves show little alteration in the presence of high magnesium, but the b-wave is almost entirely abolished[23]. The leading edge of the a-wave of the ERG is derived from photoreceptors[24], whereas the c-wave originates in the pigment epithelium[25] but depends on receptor activity for its generation[26]. The b-wave, on the other hand, arises proximally to the receptors[24,27]; its selective loss in the presence of high magnesium is to be expected if transmission between photoreceptors and second-order neurones is blocked. In some experiments, a small loss in amplitude of a- and c-waves was noted; this was probably due to the one-third reduction in Na^+ concentration in the Mg-Ringer[28]. As we have said, the effects of a drop of Mg-Ringer on the eyecup preparation are transient; the tiny b-waves seen in the responses obtained 15–20 min after the test Ringer was applied to the preparation represent the initial stages of b-wave recovery.

The likelihood that vertebrate photoreceptors secrete a depolarizing transmitter in darkness raises two questions. The first concerns the mechanisms by which bipolar cell responses of opposite polarity are produced by the action of a depolarizing transmitter. The hyperpolarizing bipolar cells exhibit an increase in membrane resistance during light stimulation[8,29], and thus generation of these responses may be similar to that of horizontal cell potentials. The depolarizing bipolar cells pose a more difficult problem. Nelson has recently found that the photic responses of these cells in the mudpuppy (*Necturus*) are accompanied by a decrease in membrane resistance[29]. If these bipolar cells also receive their input directly from receptors, this finding implies that the effect of the neurotransmitter is to decrease conductance of the cell. Although unconventional action for a neurotransmitter, recent experiments suggest that this does occur in cells of the frog sympathetic ganglion[30]. Alternatively it is possible that depolarizing bipolar cells do not receive direct synaptic input from receptors, a suggestion that has already been made by Tomita[8].

The second question concerns the identification of the photoreceptor neurotransmitter. It has been shown that certain amino-acids rapidly depolarize skate horizontal cells[17,31,32]. L-Na aspartate is especially potent in this respect (see Fig. 2 in Dowling and Ripps[17]), and might be considered as a possible candidate for the photoreceptor transmitter. It is noteworthy that recent work indicates that L-glutamate is the probable neurotransmitter at electroreceptor synapses in the skate[33].

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