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Effects of acute cooling on fish electroretinogram: A comparative study

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ABSTRACT

Temperature dependence of electroretinogram (ERG) was investigated in 3 fish species occupying different habitats — dogfish shark (*Scyliorhinus canicula*), Prussian carp (*Carassius gibelio*) and European eel (*Anguilla anguilla*). Acute cooling of the shark isolated eyecup from 23 °C down to 6 °C induced suppression of the electroretinographic b-wave — a complete degradation of this component was observed at 6 °C. On the other hand, photoreceptor component of the ERG, the negative late receptor potential was not affected by cooling. The fact that the suppression of the dogfish shark b-wave at low temperatures was as a rule irreversible testifies about breakdown of neural retinal function at cold temperature extremes. Although *in vivo* experiments on immobilized Prussian carps have never resulted in complete deterioration of the b-wave at low temperatures, significant suppression of this ERG component by cooling was detected. Suppressing the effect of low temperatures on Prussian carp ERG might be due to the fact that *C. gibelio*, as well as other cyprinids, can be characterized as a warmwater species preferring temperatures well above cold extremes. The ERG of the eel, the third examined species, exhibited the strongest resistance to extremely low temperatures. During acute cooling of *in situ* eyecup preparations of migrating silver eels from 30 °C down to 2 °C the form of ERG became wider, but the amplitude of the b-wave only slightly decreased. High tolerance of eel b-wave to cold extremes shown in our study complies with ecological data confirming eurythermia in migrating silver eels remarkably adapted to cold-water environment as well.

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1. Introduction

In their historical work on the isolated frog eye Einthoven and Jolly demonstrated that the light stimulus evokes retinal bioelectric activity leading to the formation of global eye potential electroretinogram (ERG) composed of three waves called a-, b- and c-waves (Einthoven and Jolly, 1908). Later Granit recorded the ERG from the anesthetized (by ether) cat using corneal electrodes and observed the gradual removal of the different components as the level of anesthesia was deepened (Granit, 1933). Granit defined three ERG components in order of their disappearance: P-I, P-II and P-III. He suggested that the negative a-wave is the leading edge of the negative P-III component; the positive b-wave reflects the summation of P-II and P-III while the slow positive c-wave is the summation of P-I and P-III. Pharmacological isolation of P-III can also be induced by another drug, sodium iodate ($NaJO_3$). In the number of studies it was shown that introduction of iodate into eyecup preparations causes selective suppression of P-I and P-II components, leaving completely unmasked P-III (Andjus et al., 1988, 1998b,c, 2000; Damjanović et al., 1990; Gačić et al., 2007). The most important information on the origin of ERG components was obtained from ERG recordings with intra-retinal microelectrodes (Murakami and Kaneko, 1966; Brown, 1968). These and numerous other studies indicated that the P-III component is generated as a consequence of the discharge of photoreceptors. P-III will be referred as late receptor potential (LRP) hereafter. The c-wave, which corresponds to the P-I component of Granit, is now known to originate in the pigment epithelium. By separating the retina from the pigment epithelium, c-wave can be eliminated (Dowling and Ripps, 1972). By further exposing the isolated retina to drugs, such as aspartic acid, that block synaptic transmission from the photoreceptors to the neurons in the inner nuclear layer, positive b-wave disappears and complete LRP can be unmasked and studied (Dowling and Ripps, 1972; Witkovsky et al., 1975; Peppeberg et al., 1978). This effect indicates that the b-wave originates in retinal cells that are post-synaptic to the photoreceptors. More recent studies pointed directly to the ON-center bipolar cells as generating the ERG b-wave (Sieving et al., 1994; Lei and Perlman, 1999).

Data on temperature effects on ERG components are rather scarce in literature. Prosser and Heath (1991) suggested that integrated systems are more temperature sensitive than their component parts and that the detrimental effects of high and low temperature on the brain function can be due to synaptic failure in the central nervous system. Since the

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retina can be regarded as an outgrowth of the brain, one can expect that its acute cooling down to cold extremes should primarily affect the ERG components of extrareceptor origin, especially the b-wave generated by second order retinal neurons. Preliminary evidence of suppressing effect of cold extremes on dogfish shark ERG was obtained in early work of Andjus et al. (1983). The ERG of the detached retina of the dogfish shark, after being maintained in the refrigerator for 20 h at a temperature of 4–6 °C transformed from the complex form into a completely isolated LRP. However, the issue remains open to what extent the degradation of ERG was caused by cooling and to what degree it was due to degenerative processes during preparation aging. In order to determine effects of low temperature more precisely, fish eyecup preparations were subjected to acute cooling in the present study. Effects of acute cooling on ERG components were investigated in three fish species occupying different habitats: a) bottom dwelling small spotted dogfish shark *Scyliorhinus canicula*, b) eurithermic European eel (*Anguilla anguilla*) being on the migratory, silver stadium at the early stage of seaward spawning migration and c) a more thermophylic cyprinid Prussian carp (*Carassius gibelio*). Experiments were performed on isolated eyecups of dogfish shark and *in situ* eyecup preparations of eel and Prussian carp. Temperatures of eyecups were gradually lowered from relatively high levels (25–30 °C) down to cold extremes well below 10 °C during a maximum of 4 h. The aim of the current study was to evaluate the tolerance of ERGs of examined fishes to acute cooling, and to check to what extent obtained results coincide with data regarding thermal endurance of same fishes reported in a number of ecological and physiological studies (Andjus et al., 1998a; Tesch, 2003; Aarestrup et al., 2009; Friedlander et al., 1976; Zeng et al., 2014). Special attention was focused on electroretinographic b-wave being the basic indicator of retinal neural function.

2. Materials and methods

2.1. Animals

Small-spotted dogfish sharks (*S. canicula*; 150–250 g body mass) were captured by trawler nets in the South Adriatic, at a depth of about 100 m. They were maintained at 17 °C for at least one month prior to the experiments in a sea-water recirculation system for experimental aquaculture, located in a dark and temperature controlled room. At no time were the dogfishes exposed to light for long, as this is known to be damaging to the elasmobranch photoreceptors (Hamasaki et al., 1967). Migratory European eels (*A. anguilla*) were captured using an electric gear, during summer months, in estuaries of coastal running waters along the Kotor Bay (Montenegro). They were kept subsequently, for at least 20 days prior to the experiments, in freshwater aquaria, located in a dark and temperature-controlled room, at 15 °C. Prussian carps were electrofished in the floodplain zone of the Danube River 1136 river kilometer (rkm). Fish were kept in captivity in a dark room for at least 15 days in order to acclimatize to experimental conditions. Water temperature was between 15 and 17 °C.

2.2. Preparations

Isolated eyecups excised after rapid decapitation of the dogfish shark were surgically deprived of cornea, lens, and most of the vitreous. The dogfish shark are remarkably tolerant to anoxia which allowed recording for hours from its isolated eyecups without additional perfusion. The preparation was filled with elasmobranch Ringer (Rybak, 1973) and placed in a plastic temperature controlled chamber inside a light-proof Faraday cage. The temperature within the eyecup was measured with thermistors.

Eels and Prussian carps (50–70 g body mass) were anesthetized (phenobarbital sodium) and curarized (tubocurarine) by following procedures recommended by Hamasaki et al. (1967) and by adjusting the dosage to arrest respiratory movements. Immobilized fishes were positioned laterally on a plastic platform inside a light-proof Faraday cage. Artificial respiration was provided continuously by forcing aerated and temperature-controlled water through the gills. The *in situ* eyecup preparations were surgically deprived of cornea, lens, and most of the vitreous, and filled with teleost Ringer. In previous studies it was shown that the *in situ* eyecup preparations of the immobilized eels and Prussian carps were robust enough to allow stable electroretinographic recordings for hours (Andjus et al., 1998b; Gačić et al., 2014). Long-term stable recordings were also performed from *tectum opticum* of *C. gibelio* in similar *in vivo* conditions (Damjanović et al., 2009). In other words, eels and Prussian carps were proved to be highly resistant to acute electrophysiological experiments. The temperature of the fish during acute cooling was measured from the anal orifice with thermistors. At the conclusion of experiments, fish were killed by decapitation.

2.3. Electroretinography

ERG potentials were detected with nonpolarizable chlorided silver ($Ag–AgCl_2$) electrodes, the active one placed in the interior of the saline filled eyecup. The reference electrode was in contact with the retro-orbital space behind the eyecup preparation. Electrodes were connected to the input stage of a directly coupled differential preamplifier, and responses were recorded from a storage oscilloscope display using a Polaroid camera. Photic stimuli were delivered by a single-beam optical system using an 8-V, 50-W tungsten-halogen lamp as the light source, and providing independent control of intensity (neutral density filters), duration (electromagnetic shutter), and spectral composition (interference filters) of the test flashes. A heat filter virtually eliminated wavelengths >700 nm. The stimuli consisted of single flashes guided through a fiber optic cable positioned normal to the surface of the eyecup. The flashes casted a circular patch of light just covering the external surface of the preparation. The duration of the light stimulus was 200 ms in all experiments. Intervals between test flashes were kept sufficiently long so as not to influence subsequent responses. Light intensities were calibrated and checked by placing the active surface of the radiometer probe in the position usually occupied by the eyecup preparation. Unattenuated, the energy flux delivered by the test field was of the order of 2×10^{-2} mW/cm^2.

The procedure used for testing of temperature effects on dogfish shark ERGs was as follows. Dogfish sharks were kept for at least 20 days prior to the experiments, in seawater aquaria, located in a dark and temperature-controlled room, at 17 °C. In the first step of the experiment temperature of fish eyecup preparation was raised to 25 °C and kept at this level for half an hour. Before the temperature was decreased to subsequent intermediate level control ERG was recorded. Afterwards eyecup temperature was gradually decreased, and at each transitional temperature the same procedure was repeated.

Both, eels and Prussian carps were kept for at least 20 days prior to the experiments, in freshwater aquaria, located in a dark and temperature-controlled room, at 15 °C. In the first step of the experiment body temperature was raised to 30 °C in eel and 25 °C in *C. gibelio* and kept at these levels for half an hour. Afterwards fish body temperature was gradually decreased in the similar manner as shown in the shark.

2.4. The ocular index

The silver (migratory) stage of eels used in the present experiments was specified on the basis of the eye index, *I*, according to Pankhurst (1982):

$$I = \left[\frac{\left(\frac{A+B}{4}\right)^2 \cdot \pi}{L}\right] \cdot 100$$

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