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Retinal electrophysiology for toxicology studies: Applications and limits of ERG in animals and ex vivo recordings

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Abstract

Assessing retinal drug toxicity is becoming increasingly important as different molecules are now developed for the treatment of neurodegenerative diseases and vascular disorders. In pharmacology and toxicology, the electroretinogram (ERG) and the multielectrode array (MEA) recording techniques can be used to quantify the possible side effects of retino-active xenobiotics. Toxicity testing requires the use of rodent as well as non-rodent models for extrapolation to the human model when determining risk and safety. Animal species differ in their retinal anatomo-physiology: most rodents used in toxicology studies are essentially nocturnal species, whereas the non-rodent laboratory species normally used (e.g. dogs, pigs and monkeys) are diurnal. The ratio between the photoreceptor populations which varies from species to species, should be considered when designing the experiment protocol and the interpretation.

The described ERG procedures are designed to comply with all applicable good laboratory practice standards. Use of these procedures should yield an acceptable level of intra- and inter-subject variability for compiling a historical database, and for detecting possible *retinal toxicity* in animal studies. They could therefore be used as specific and standardized tools for screening of potential retinotoxic molecules in drug discovery and development in order to compare methods and results with those obtained in human electrophysiological assessments. Recording of ganglion cell light responses on ex vivo retina with the MEA technique can further demonstrate how retino-active xenobiotics affect retinal visual information processing by eliminating potential obstacles related to bioavailability and blood barrier permeability.

Keywords: Electroretinogram; Toxicology; Animals; Retina; Cones; Rods; Multielectrode array

Introduction

Assessing retinal drug toxicity is becoming increasingly important as different molecules are now developed for the treatment of neurodegenerative diseases and vascular disorders. In animals, behavioral assessment of visual function is not always easily obtained. Electrophysiolo-

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gical testing is an effective and objective method to assess the status of the visual pathway (Brigell et al., 2005). In pharmacology and toxicology, the electroretinogram (ERG) is used to quantify possible side effects of retino-active xenobiotics (Schaeppi et al., 1987, 1988; Gelatt et al., 2001; Shaikh et al., 2003; Tzekov et al., 2004). This retinal electrical response to a flash of light is simply recorded at the corneal surface. It is generated by radial currents that arise either directly from retinal neurons or as a result of the effect on retinal glia of changes in extracellular potassium concentration brought about by the activity of these neurons. This ERG response is an excellent tool for studying retinal function in both clinical and experimental studies because it can be recorded easily and non-invasively with a corneal electrode in intact subjects under physiological or anaesthetized conditions. However, the ERG is a gross potential that reflects the summed activity of all of the cells in the retina. For the ERG to be an effective tool in assessing normal and pathological retinal cell activity or to investigate drug toxicity, it is important to understand the respective contributions of the different retinal cells.

As it will be described in this paper, information on retinal ganglion cell (RGC) function is very difficult to assess in vivo by ERG measurements. Visual-evoked potentials (VEP) measured on the brain provide a very indirect manner and they are very difficult to quantify. Therefore, it appeared important to develop a technique to measure RGC activity. The multielectrode array (MEA) technique was shown to allow investigators to measure RGC light responses on the isolated retina. Therefore, it provides a means to assess the function of these cells after long drug treatments or to use them as biological probe for retinal information processing in the presence or absence of a perfused drug. A main advantage of this technique with respect to the in vivo approaches is the possibility to exactly control the drug concentration in the medium bathing the isolated retina.

The purpose of this paper is to provide the reader with a working knowledge of functional testing of the retina by describing the most common procedure to evaluate the retinal function in various animals species (nocturnal and diurnal) used in statutory toxicological studies. Applications and limits of these in vivo techniques will be discussed while the MEA technique will be described to illustrate its potency for toxicological studies by measuring ganglion cell light responses on ex vivo retina during bath application of tested molecules.

Conditions for generating the ERG

General overview of electroretinography

The ERG represents the electrical response that is generated by the entire retina when stimulated by a brief stimulus of light (flash or flicker) or by a brief variation of spatial organization of luminance (checkerboards where the blocks alternate, i.e. pattern). It is often compared to electrocardiogram (ECG) in that it is similarly composed of a series of waves that are presumed to originate from different retinal cells. However, unlike the ECG, which represents the ongoing activity of the myocardium, one must stimulate the retina with light to induce a synchronized retinal cell activity leading thereby to the generation of the ERG response. This concept is of extreme importance if one wishes not only to understand the origin of the ERG but also appreciate the need to normalize the ERG acquisition parameters in order to extract as much meaningful functional information as possible. For the ECG, it is the electrode position that is critical for inter-laboratory comparisons whereas with the ERG it is the stimulating parameters that are of the utmost importance. One must also take into consideration the unique physiology of the retina, which permits it to work under a wide range of luminance levels. This is for the great part due to the two types of photoreceptors: rods (for night time or scotopic vision) and cones (for daytime or photopic vision), which are found in retinas of most higher vertebrates. Furthermore, the ratio of these photoreceptor populations varies from species to species and it must be taken into consideration in the design of stimulus conditions. One of the aims of the electro-retinography is to separate the cone ERG from the rod ERG in order to distinctly assess the function of each type of photoreceptors and their respective pathways. Another factor that affects a cell's contribution to the ERG is its orientation in the retina. Because it is the radial components of the extracellular current that gives rise to the radial potential differences that are sensed in the commonly neurons (photoreceptors, bipolar cells) and glial cells (Müller cells and retinal pigment epithelial (RPE) cells) should make larger contributions to the ERG than cells that are oriented more irregularly or tangentially (e.g. horizontal and amacrine cells). Current enters the extracellular space at one retinal depth (the current source) and it returns into the cell at another depth (the current sink), creating a current dipole. ERG polarity and amplitude are critically dependent on where the active and reference electrodes are placed in this circuit. A common placement for the active electrode in non-invasive studies is on the cornea or as close as possible to the cornea. The reference electrode can be positioned anywhere in the path but is often either retrobulbar (e.g. needle inserted) or remote from the eye (e.g. at the junction between the ear and the temple). The contribution of RPE cells to the ERG are more delayed than neuronal responses because the responses of these cells depend on relatively slow changes in extracellular potassium concentration (which represents the indirect effect of light on retinal glia brought about