The Electroretinogram as a Biomarker of Central Dopamine and Serotonin: Potential Relevance to Psychiatric Disorders

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Background: Dysfunctions in brain dopamine and serotonin neurotransmission are believed to be involved in the etiology of psychiatric disorders, and electroretinogram (ERG) anomalies have been reported in psychiatric patients. The goal of this study was to evaluate whether ERG anomalies could result from central dopamine or serotonin dysfunctions or from changes in the retinal bioavailability of these neurotransmitters.

Method: Photopic and scotopic ERGs were recorded in R439H tryptophan hydroxylase 2 knockin (Tph2-KI) mice that have an approximately 80% decrease in brain serotonin and dopamine transporter knockout (DAT-KO) mice showing a fivefold increase in brain extracellular dopamine. Dopamine and serotonin retinal and striatal tissue content were also measured. The role of dopamine D1 receptors (D1R) and D2 receptors (D2R) in the ERG responses was evaluated in D1R-KO and D2R-KO mice.

Results: An increase in photopic b-wave implicit time was observed in Tph2-KI mice (wildtype = 24.25 msec, KI = 25.22 msec; $p = .011$). The DAT-KO mice showed a decrease in rod sensitivity (wildtype = −1.97 log units, KO = −1.81 log units; $p = .014$). In contrast to remarkable alterations in brain levels, no changes in dopamine and serotonin retinal content were found in DAT-KO and Tph2-KI mice, respectively. The D1R-KO mice showed anomalies in photopic and scotopic maximal amplitude, whereas D2R-KO mice showed higher oscillatory potentials relative contribution to the b-wave amplitude.

Conclusion: Alterations in central dopamine and serotonin neurotransmission can affect the ERG responses. The ERG anomalies reported in psychiatric disorders might serve as biomarkers of central monoaminergic dysfunction, thus promoting ERG measurements as a useful tool in psychiatric research.

Key Words: Biomarker, dopamine, dopamine transporter, electroretinogram, psychiatric disorders, serotonin

Understanding of the biological underpinnings of psychiatric disorders remains elusive in part due to well-known limitations in approaches used to investigate the living human brain. There is a strong demand to develop novel approaches to study human brain functions indirectly. One interesting subject of such studies is the retina, because it is a part of the central nervous system, and thus changes in retinal function might reflect anomalies found in brain disorders. These measurements can be achieved with electroretinogram (ERG) recordings, a noninvasive technique to monitor the light-evoked electric potential originating from the retina in response to standardized flash stimuli. Multiple studies reported retinal anomalies as measured with the ERG in patients with psychiatric or neurologic disorders such as schizophrenia (1,2), seasonal affective disorder (SAD) (3–6), autism (7), and Parkinson's disease (8) as well as in young offspring at high risk to develop schizophrenia or bipolar disorder (9). However, whether these ERG anomalies are related to neurotransmission dysfunctions in the retina or in the central nervous system remains unknown. It should be noted that, although brain dopamine and serotonin are known to be involved in psychiatric disorders, these two neurotransmitters also play important roles in retinal physiology.

Dopamine is the major catecholamine expressed in the retina of most species (10). A subtype of amacrine cells located in the proximal part of the inner nuclear layer releases dopamine, which is known to play a role in light adaptation (11). Five dopaminergic receptors (D1R to D5R) grouped into two sub-classes (D1-class and D2-class) according to pharmacological properties and intracellular signaling responses have been described (12). In the mammalian retina, D1R are expressed in bipolar, ganglion, and horizontal cells. The D1-class receptors are responsible for the uncoupling of horizontal and amacrine cell-gap junction (13). The D2R are found in the synapse between horizontal cells, bipolar cells, and photoreceptors (14). Dopaminergic cells in the brain are known to express the dopamine transporter (DAT), which regulates extracellular dopamine concentration by mediating its reuptake and maintains proper intracellular dopamine stores (15). Thus, in mice lacking the DAT (DAT-knockout [KO] mice) extracellular levels of dopamine in the striatum are increased by 5-fold (16), whereas dopamine tissue content that is mostly reflective of its intraneuronal storage is decreased by 20-fold (17).

Serotonin is another monoaminergic neurotransmitter found in the retina of mammals but at a lower level than dopamine (18). Tryptophan hydroxylase 2 (Tph2) is the rate-limiting enzyme for neuronal serotonin synthesis (19,20), whereas the closely related enzyme Tph1 is responsible for serotonin synthesis in peripheral

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Received Jul 6, 2012; revised Nov 26, 2012; accepted Nov 27, 2012.

0006-3223/$36.00
http://dx.doi.org/10.1016/j.biopsych.2012.11.024

tissues and the retina (21). Anatomical studies also demonstrated that mammalian retinas receive inputs from the brain via retinopetal axons emerging from the optic nerve (18). Recently, a rare human variant (R441H) of Tph2 was identified in few individuals with unipolar major depression (22). On the basis of this mutation, Tph2 knockin (Tph2-KI) mice carrying the equivalent R439H mutation were generated. These mice display a reduction of approximately 80% brain serotonin synthesis and express depression-related behavioral abnormalities (23) and peripheral biomarkers (24).

Several pharmacological studies (25–30) suggest that alterations in dopamine and serotonin transmission can modulate the ERG responses, but the particular contribution of retinal or central serotonin- and dopamine-related processes in the ERG modulation is still unknown. The goal of this study was to evaluate whether the ERG anomalies similar to those reported in patients with psychiatric disorders can be related to central dopamine or serotonin dysfunctions or to alterations in retinal concentrations of these neurotransmitters. Both photopic and scotopic ERGs were assessed in Tph2-KI mice to investigate the impact of decreased brain serotonin on the ERG and in DAT-KO mice to evaluate effects of persistently elevated dopaminergic tone in the brain (16). We also investigated the role of dopamine D1R and D2R receptors in ERG responses with respective KO mice to evaluate whether abnormalities found in these mutants could be implicated in ERG anomalies described in psychiatric disorders.

Methods and Materials

Animals

Generation of DAT-KO (31), D1R-KO (32), and Tph2-KI (23) mice has been described previously. The D2R-KO mice (33) were obtained from The Jackson Laboratory (Bar Harbor, Maine). Mice were genotyped at weaning by polymerase chain reaction amplification from ear punch biopsy of genomic DNA. All genotypes were reconfirmed by additional genotyping after experimentation. For all experiments, respective wildtype (WT) littermates were used as control subjects for homozygous mutant mice, and all animals (males and females) were 3 to 5 months of age. Mice were housed 4–5/cage in a humidity-controlled room at 23°C on a 12-hour light-dark cycle with ad libitum access to food and water, before experiments. The Université Laval Institutional Animal Care Committee approved all experimental procedures in line with guidelines from the Canadian Council on Animal Care.

ERG Recording

Animals were anesthetized with a mixture of ketamine (80 mg/kg) and xylazine (10 mg/kg) injected intraperitoneally, and body temperature was maintained between 36°C and 38°C with a homeothermic blanket during the ERG recording. Only the left eye was recorded. The pupil was dilated with a drop of 1% tropicamide (Mydriacyl, Alcon Canada, Mississauga, Ontario, Canada) applied on the corneal surface, which was anesthetized with a drop of .5% proparacaine hydrochloride (Alcaine, Alcon Mississauga, Ontario, Canada). Recordings were obtained with a DTL electrode (Shieldex 33/9 Thread; Statex, Bremen, Germany) shaped into a loop and placed on the corneal surface. Ground and reference electrodes (Grass Technologies, Astro-Med, Brossard, Quebec, Canada) were placed in the tail and in the mouth, respectively. Flash stimulations were administered with white light generated by light-emitting diodes (for scotopic ERG) and by xenon flashes (for photopic ERG) housed in the Ganzfeld Color Dome (Espion, Diagnosys, Littleton, Massachusetts) to achieve full retinal stimulation. Photopic and scotopic ERGs were performed on different mice.

Scotopic ERG was used to assess rod function. After overnight dark adaptation, animals were prepared for ERG recording under a dim red light. Scotopic luminance-response function was obtained with 10 increasing single white flash stimulus strengths ranging from −3.62 to .46 log cd × sec/m^2, with interstimuli interval set at 15 sec (first 4 intensities) and 30 sec (last 6 intensities). Photopic ERG was used to assess cone function. Animals were light-adapted for 10 min to a light background set at 30 cd/m^2. A photopic luminance-response function was generated with 9 stimulus strengths of brief flashes, ranging from −.81 to 2.86 log cd × sec/m^2, with an interstimuli interval set at 15 sec (first 3 intensities) and 30 sec (last 6 intensities). In both scotopic and photopic condition, for each stimulus strength, at least 4 responses or more were averaged to achieve a high signal-to-noise ratio and confirm the reproducibility of the response.

Data Analysis

The ERG response is primarily composed of a negative wave called the a-wave, followed by a positive component, the b-wave. The a-wave is generated mainly by the photoreceptors, whereas the b-wave originates from the bipolar cells. By convention, a-wave amplitude is measured from the baseline to trough, and the b-wave is measured from the trough of the a-wave to the peak of the b-wave (Figure 1). On the ascending part of the b-wave, the oscillatory potentials (OPs) can be observed. Although their exact origin is still unclear, it is believed that they could result from the interaction between bipolar cells, amacrine cells, and ganglion cells (34).

As per Hébert *et al.* (35), the b-wave amplitudes were plotted against flash luminance to generate the photopic and scotopic luminance response function from which two parameters were derived, namely Vmax and logK. Vmax refers to the maximal b-wave amplitude where the system saturation occurs. In scotopic condition, Vmax was selected at the end of the plateau before cones intrusion. LogK is interpreted as retinal sensitivity and represents the intensity necessary to reach half of the Vmax. In the current study, to avoid some subjectivity in the selection of the Vmax parameter individually, the Vmax was set at 2.86 log cd × sec/m^2 in photopic condition and at −.0105 log cd × sec/m^2 in scotopic condition. The latter intensities were selected after visual inspection of the averaged luminance response functions in a group of 10 WT mice. As a complimentary measure for retinal sensitivity, the criterion threshold intensity (T50 μV) was calculated. This criterion is also derived from the luminance-response function and represents the flash luminance necessary to reach a b-wave amplitude of 50 μV (4).

The OPs were directly extracted from ERG recording by the Espion software in photopic condition or digitally extracted with a Butterworth filter (bandpass: 75–300 Hz, attenuation: −6 dB) with MATLAB R2012a (MathWorks, Natick, Massachusetts) in scotopic condition. The sum of OPs was used for analysis. Because OPs are components of the b-wave, the ratio Vmax/sum of OPs amplitude was also used to provide a relative measure of OPs contribution to the b-wave amplitude.

Repeated-measures analyses of variance were performed to assess the difference between WT and homozygous mutant mice in all the ERG parameters (a- and b-wave amplitude and implicit time) in photopic and scotopic conditions. Analysis of the ERG also included the logK as well as the value of the ERG parameters observed at the Vmax, including OPs. The later variables were