Vision Res. Vol. 24, No. 1, pp. 87–90, 1984
Printed in Great Britain.

0042-6989/84 $3.00 + 0.00

RESEARCH NOTE

ROD SATURATION IN *b*-WAVE OF THE RAT ELECTRORETINOGRAM UNDER TWO DIFFERENT ANESTHETICS

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(*Received* 2 *April* 1982; *in revised form* 13 *April* 1983)

Abstract—B-wave increment threshold experiments in the rat show that "rod saturation" occurs at different background levels with different anesthetics. Rod saturation builds up over the first 60 sec of light adaptation in pentobarbital anesthetized but not in urethane anesthetized animals. These and other findings suggest that "rod saturation" can occur when the rod photoreceptors themselves are not saturated.

Rod saturation Rat retina Erg *b*-wave Anesthetics

INTRODUCTION

The *b*-wave of the rat electroretinogram (erg) has been used as an index of visual sensitivity. Similar laws appear to govern rat thresholds and human rod thresholds (Dodt and Echte, 1961; Dowling, 1963; Massof and Jones, 1972; Ernst and Kemp, 1975; Birch and Jacobs, 1975; Cicerone, 1976). About 10 years ago, Green (1971; 1973) conducted *b*-wave increment threshold experiments with the two color threshold method of Aguilar and Stiles (1954) and obtained evidence of "rod saturation" and a Purkinje shift. Rod saturation is an upward deviation from the Weber–Fechner line of the curve relating increment threshold to adapting background intensity (see Fig. 1b). Thresholds with backgrounds above this critical "rod saturation" level were determined by cones.

Recently, in the course of doing other studies, we discovered that "rod saturation" is anesthetic dependent. That is one obtains different results depending on whether pentobarbital or urethane anesthesia is used. This report describes the effects of pentobarbital and urethane on "rod saturation".

METHODS

Experiments were conducted on 23 albino Sprague–Dawley rats (250–350 g in weight). All the animals but one were born in our colony room, and raised in the dark, only occasionally experiencing dim red illumination. Subjects were anesthetized with interperitoneal injections of either urethane (200 mg/100 g) or sodium pentobarbital (40 mg/kg). The eyelid of the experimental eye was drawn back and sutured. The cornea was anesthetized with 1/2% Tetracaine. The pupil was dilated with atropine sulfate (1% solution). The test and adapting lights were generated by 100W solid filament tungsten (GE) and 150W xenon arc (Osram) lamps, respectively. The beams of the two lamps were optically superimposed and projected uniformly on the surface of a ping-pong ball. The intensity and spectral composition of the lights were controlled by calibrated neutral density filters and interference filters. The duration of the stimuli were controlled by a small laboratory computer (Data General Nova 2) interfaced to electromagnetic shutters (Uniblitz). A small incision was made on the side of the nose and cotton wick Ag–AgCl electrodes were placed on the cornea and at the site of the incision. The section of ping-pong ball placed over the eye acted as a diffusing screen. The body of the rat was wrapped in a heating pad, and body temperature was kept at 38°C by monitoring with a rectal probe. Each rat was anesthetized and prepared for experimentation under dim red illumination. The subject was then dark adapted for 10–15 min prior to the presentation of stimuli.

The erg signals were amplified, filtered (bandwidth 0.1 to 100 Hz), and then displayed on a storage oscilloscope (Tektronix 565). At any level of the adapting stimulus, the intensity of the test flash (200 msec duration) was adjusted to produce a criterian 50 μV response. Testing was conducted in an ascending order of adapting luminances, usually over a 3 or 4 log unit range.

RESULTS

A typical result of the increment threshold experiment on an animal anesthetized with pentobarbital is shown in Fig. 1. The open circles give the *b*-wave

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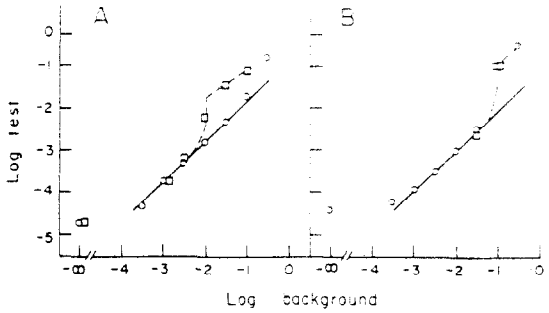

Fig. 1. Increment threshold curves for a blue-green test stimulus (500 nm) flashed on a red adapting background (Wratten 26) that has been presented for 1 sec (○) or for 60 sec (□). Each point represents a single determination of the relative energy required to produce a 50 μV *b*-wave response. Repeated measurements were always within a 0.1 log of each other. (a) The rat was anesthetized with pentobarbital (40 mg/kg). (b) Results from the same rat anesthetized with urethane (200 mg/100 g).

thresholds for a blue test flash after 1 sec of light adaptation with a red background. With the brief adapting period the Weber–Fechner relationship (the solid line in the figure) held over most of the range of luminances studied, with an upturn against the highest (−1.0) background. However, if the background remained on the sensitivity of the *b*-wave to the test flash changed dramatically. The open squares in Fig. 1(a), show that at higher backgrounds, following 60 sec of adaptation, the threshold of the blue test flash was shifted vertically approximately 1 log unit from the Weber–Fechner line.

Figure 1(b) presents typical increment threshold data under urethane anesthesia (200 mg/100 g). In contrast to the results in Fig. 1(a), rod saturation may be seen in the 1 sec condition. At the background of −1 the threshold was shifted vertically 1 log unit from the Weber–Fechner line. The thresholds at 1 sec and 60 sec were virtually the same. Figure 2 shows the results of using both a blue-green and a red test flash to probe sensitivity. A Purkinje shift occurs at background intensities above rod saturation. Figure 3 illustrates

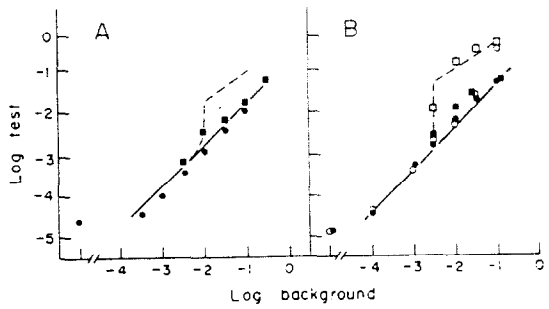

Fig. 2. Increment threshold curves for a 500 nm test (open symbols) and a 650 nm (solid symbols) on a red adapting background. Rats anesthetized with pentobarbital. (a) Same animal as in Fig. 1. Smooth curves redrawn from Fig. 1 indicate trend of 500 nm test data. Measurements with 650 nm test at 1 sec (●) and 60 sec (■) are shown. (b) Complete set of measurements with 500 and 650 nm tests at 1 sec (○,●) and 60 sec (□,■) on another animal.

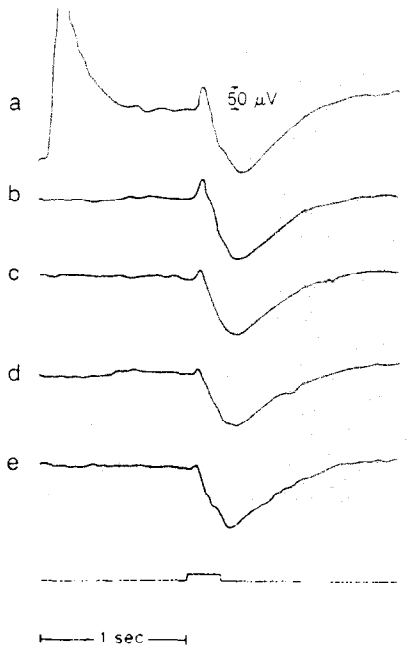

Fig. 3. Time-course of *b*-wave saturation under pentobarbital anesthesia. Recorder tracings of responses to flashes at various times. The blue-green test flash (−2.0 log) was presented every 12 sec. The red adapting background (−2.0 log) was turned on at the initiation of trace (a), and the first test flash was presented one second later. Traces (b–e) show responses at 13–49 sec after onset of adaptation.

the time-course of the development of saturation under pentobarbital anesthesia. In trace (a), the adapting background light was turned on and one second later the 200 msec test flash was presented. As seen in this trace, the test flash produced a criterion 50 μV *b*-wave without an *a*-wave, but with a prominent PIII wave (the large negative potential). In traces (b–e) the adapting background remained on continuously and the test flash was successively presented in 12 sec intervals. The amplitude of the *b*-wave diminished over time from nearly 50 μV in (a) to less than 10 μV in trace (e). This change in *b*-wave amplitude occurred in the 49 sec interval following the onset of the adapting background. In contrast PIII was not affected by the duration of adaptation. Under urethane, the amplitude of the *b*-wave following 1 sec of light adaptation was the same as that following 60 sec. This appeared to hold irrespective of the intensity of the adapting light over the 4 log unit range of backgrounds we have studied (records not shown).

DISCUSSION

These findings depart from Green's (1971, 1973) observation that rod saturation occurred at about the same intensity after 1 sec adaptation as after steady state adaptation. One important difference is the anesthetic. Green's earlier studies were performed under urethane anesthesia rather than with pentobarbital.

Consequently, we decided to repeat the experiment using urethane anesthesia. As soon as we switched anesthetics we obtained results indicating that this was an important variable. The differences between Fig. 1(a) and (b) seem to be due to differences in the action of different anesthetics on retinal function. This conclusion was completely confirmed by making measurements in five animals using both anesthetics. Because of the very long lasting effects of urethane in all but one instance animals were first anesthetized with pentobarbital and then, after complete recovery, anesthetized with urethane. The one exception was an animal given urethane first and then pentobarbital after it had recovered (2 days later). Results like those in Fig. 1(a) were obtained with pentobarbital (on these 5 as well as on another 10 animals) and like those of Fig. 1(b) with urethane (7 animals total). That is, under pentobarbital anesthesia rod saturation not evident after 1 sec was seen after 60 sec of adaptation. Under urethane anesthesia, rod saturation occurred in the 1 sec condition, though usually at background levels about 1 log unit greater than that required for saturation in the 60 sec condition with pentobarbital. After 60 sec of adaptation with both anesthetics, the threshold remained virtually unchanged, even after an additional 5 min of adaptation (Green, 1973). While the dark adapted threshold for the pentobarbital animal in Fig. 1a is slightly lower than that for the urethane animal (Fig. 1b), this was not a consistent difference. For example, the average dark adapted threshold for the five animals given both drugs was -4.66 ± 0.14 SE with pentobarbital and -4.58 ± 0.13 SE with urethane. Since in most instances animals were given pentobarbital and then urethane it seemed possible that the above differences might be due to the sequence of administration. It is well known that the initial light exposure to dark-reared animals leads to massive disk shedding. Such shedding could well influence the outcome with urethane and could have led to the higher intensity background required for *b*-wave saturation. To control for this, as mentioned above, we did one animal in reverse order. In addition, 3 animals were done twice in succession with pentobarbital. The pattern of results in these animals was dependent on anesthetic and not on sequence of administration. These observations show that rod saturation, is dependent upon the choice of anesthesia.

Thus, "rod saturation" builds up over the first 60 sec of adaption in pentobarbital anesthetized animals, but not in urethane anesthetized animals. What are the physiological changes producing the anesthesia differences? Three different classes of hypotheses may account for the influence of anesthesia on rod saturation: (1) direct differential effects of the anesthetics on the photoreceptors themselves; (2) drug dependent scotopic–photopic interactions; (3) post-receptor drug effects. With pentobarbital and 60 sec of adaptation, the rods saturate at a background approximately 2.0 log units below full intensity. This background corresponds to about 100 quanta absorbed rod^{-1} sec^{-1}. A level below the 500–1000 quanta rod^{-1} sec^{-1} reported to saturate rod photoreceptors in the rat (Penn and Hagins, 1972). In contrast, rod saturation occurred at about 1000 quanta rod^{-1} sec^{-1} in urethanized animals. The above seems to indicate that these anesthetics are not directly affecting the photoreceptors themselves. Consistent with this, we find under pentobarbital a build-up of *b*-wave desensitization and little or no effect on PIII.

To establish the class of photoreceptors mediating sensitivity in these experiments, we measured spectral sensitivity at 500 and at 650 nm. The results (see Fig. 2) show, for pentobarbital animals, what had been previously demonstrated using urethane (Green, 1971, 1973). From absolute threshold up to background intensities that saturate, the responses have rod spectral sensitivity. Furthermore, under all conditions, following saturation the mechanism determining sensitivity is *not* scotopic; there is a Purkinje shift. Hence, rod photoreceptors determine threshold until the background intensity is saturating, at which point the photopic mechanism takes over.

In an attempt to understand the physiological basis of rod saturation, Green (1973) compared *a*- and *b*-wave increment thresholds. As Dowling (1967) had previously reported, *a*-wave increment thresholds were unaffected by low level backgrounds (see Green and Powers, 1982) and were a steeply rising function of background intensity at moderate levels of background intensity. Green added the observation that the *b*-wave saturated at about the same background intensity that caused the *a*-wave increments to turn sharply upward. He concluded from this that rod saturation, as expressed in the *b*-wave, was a property of the rod photoreceptors themselves. That is, saturation of *b*-wave increment thresholds results from the background increasing the rod signal so close to its maximum level that the increment generated by a test flash becomes negligibly small. In the present study, we have re-examined rod saturation using the two-color threshold method and report new observations which are inconsistent with Green's earlier suggestion.

These new findings indicate that "rod saturation" of the *b*-wave may occur at background levels where the photoreceptors are not saturated. This type of phenomenon has been reported in skate retina (Green *et al.*, 1975), but was not known to occur in the rat. Lennie *et al.* (1975) have studied rod saturation as expressed in the ganglion cells of the urethane anesthesized cat. Increment thresholds for small, brief flashes were measured against small and large adapting backgrounds. They found that the signals controlling saturation were pooled over retinal areas which are considerably larger than individual rods and approached the size of the ganglion cell receptive field centers. A neural mechanism that pools signals for saturation, but not at the level of the rods themselves, could explain the present results (hypothesis 3). Recent experiments on man have indicated time dependent

changes in psychophysically determined rod saturation. That is, rods saturate at lower intensities with flashed backgrounds compared to those with steady backgrounds (Adelson, 1977, 1982; Geisler, 1979). Our findings with pentobarbital are in the opposite direction—saturation occurred at higher intensities against flashed backgrounds than with steady-state backgrounds.

Acknowledgements—Adrienne Graves and Peter Lukasiewicz read an earlier manuscript and offered helpful suggestions. This research was supported by NIH grant EY00379 to D.G.G.

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