Influence of anesthetics, ethyl alcohol, and Freon on dark adaptation of monkey cone ERG. Dirk van Norren and Pieter Padmos.

Cone dark adaptation curves were measured in a rhesus monkey using the electroretinogram (ERG) response to a 40 Hz flickering stimulus. The influence of anesthetics on the time course of dark adaptation was studied. All volatile anesthetics tested (methoxyflurane, halothane, enflurane, ether, chloroform) retarded dark adaptation but to different degrees; urethane, ethyl alcohol, and Freon 11 also retarded dark adaptation. No effect was found for barbiturates and ketamine. It seems unlikely that metabolites play a role in the observed phenomena. A literature survey reveals that several studies on dark adaptation or visual pigment regeneration might have suffered from influences of the anesthetic used. The cause of the phenomenon might lie either in anesthetics-induced membrane changes or in hindrance of the isomerization of 11-trans retinal to 11-cis retinal.

Recently we described that the general anesthetic halothane retarded cone dark adaptation (DA) in human subjects, macaque monkeys, and birds. Although we used a measuring method based on the electroretinogram (ERG), it was speculated that the origin of the retardation would lie in a retarded regeneration of the visual pigment. This has now been confirmed by retinal densitometry. The present study was undertaken to test a series of commonly used general anesthetics with regard to their influence on cone DA.

Methods. Stimulus and recording methods were identical to the ones described by Norren and Padmos. The corneal ERG to a yellow (\( \lambda = 577 \) nm.), 45 deg. stimulus was kept, by means of a feedback on the light intensity, at a constant level (1 \( \mu V \)). A continuous record of the light intensity provided a measure of the retina’s sensitivity. The bleaching light consisted of a 70 deg., 6.2 log td. yellow light (Schott OG 550) presented during 5 to 10 min. Dark adaptation curves, representing the recovery of the retina’s sensitivity after the bleaching light was extinguished, resembled exponential functions, and therefore, the rate of DA could be characterized by a single datum: the time constant (1/e value).

The subjects were rhesus monkeys of between 2.5 and 6 kilograms. The monkeys received phencyclidine, 0.4 mg. per kilogram (Table 1) intramuscularly (I.M.), or 10 mg./kg. of ketamine I.M. as sedation. Intravenous (I.V.) injection of 1 to 2 mg./kg. of methohexital, a short-acting barbiturate, allowed further preparation of the animal. After intubation the animal received an initial I.V. dose of 30 mg./kg. of the muscle relaxant pancuronium bromide, followed by a continuous injection of 30 mg./kg. per hour of the same agent. Artificial respiration was then started. The gas mixture consisted of 70 per cent N,O and 30 per cent O, entered the gas mixture. In the rest of the report this is referred to as “the basic anesthesia.” The N,O was replaced by oxygen only on rare occasions in order to avoid stress for the animal. The supply was regulated such that expiratory CO was kept between 4.0 and 5.5 per cent. At the beginning of the experiment and about 4 hours later 0.1 mg./kg. of atropine sulfate was given to suppress salivation.

REFERENCES
Table I. List of agents used

<table>
<thead>
<tr>
<th>Name</th>
<th>Trade name</th>
<th>Concentration</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acepromazine</td>
<td>Vetranquil</td>
<td>10 mg/ml.</td>
<td>Philips-Duphar, Amsterdam, The Netherlands</td>
</tr>
<tr>
<td>Atropine sulfate</td>
<td>—</td>
<td>0.5 mg/ml.</td>
<td>Brocades, Amsterdam, The Netherlands</td>
</tr>
<tr>
<td>Enflurane</td>
<td>Ethrane</td>
<td>99.9%</td>
<td>Abbott, Brussels, Belgium</td>
</tr>
<tr>
<td>Halothane</td>
<td>Halothane</td>
<td>99.9%</td>
<td>Hoechst, Frankfurt, Germany</td>
</tr>
<tr>
<td>Ketamine</td>
<td>Ketalar</td>
<td>50 mg/ml.</td>
<td>Parke-Davis, Madrid, Spain</td>
</tr>
<tr>
<td>Methohexital</td>
<td>Brevital</td>
<td>10 mg/ml.</td>
<td>Lilly, Indianapolis, U.S.A.</td>
</tr>
<tr>
<td>Methoxyflurane</td>
<td>Penthrane</td>
<td>99.9%</td>
<td>Abbott, Brussels, Belgium</td>
</tr>
<tr>
<td>Pancuronium bromide</td>
<td>Pavulon</td>
<td>2 mg/ml.</td>
<td>Organon, Oss, The Netherlands</td>
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<tr>
<td>Pentobarbital</td>
<td>Nembutil</td>
<td>60 mg/ml.</td>
<td>Abbott, Brussels, Belgium</td>
</tr>
<tr>
<td>Phencyclidine</td>
<td>Sernylan</td>
<td>20 mg/ml.</td>
<td>Parke-Davis, London, England</td>
</tr>
<tr>
<td>Thiopental</td>
<td>Pentothal</td>
<td>50 mg/ml.</td>
<td>Abbott, Brussels, Belgium</td>
</tr>
<tr>
<td>Trichlorofluoromethane</td>
<td>Freon II</td>
<td>&gt;99.9%</td>
<td>DuPont, Geneva, Switzerland</td>
</tr>
<tr>
<td>Urethane</td>
<td>—</td>
<td>Crystalline powder</td>
<td>Brocades, Amsterdam, The Netherlands</td>
</tr>
</tbody>
</table>

Results. The retardation effect of five inhalation anesthetics on cone DA as a function of the concentration is plotted in Fig. 1. The time constant, plotted on the ordinate, is a measure of the rate of cone DA. We expressed the concentration of the anesthetics in multiples of the Minimum Alveolar Concentration (MAC). The MAC is a standard for anesthetic potency defined for human subjects, but in our experience it was also roughly valid for rhesus monkeys. Methoxyflurane was found to have the strongest effect on DA. At 0.5 MAC the time constant was about four times longer than normal, whereas the weakest of the group, enflurane, caused a change of not more than a factor of 1.5 at this MAC value.

With enflurane and ether, short (10 sec.) full bleaches were also given besides the routine 5 to 10 min. bleaches. As with halothane, a fast recovery was found after a short bleach whereas only the long bleach caused retardation; this suggests that all members of the tested group probably affect the pigment regeneration process in the same peculiar way as previously described for halothane.

The effect of injecting I.V. a 30 per cent solution of urethane on the time course of cone DA is displayed in Fig. 2, A. It shows that although the retardation effect is significant, it is less pronounced than that of most volatile anesthetics. The maximum dose of 1 gram/kg, did not bring about general anesthesia in the monkey. If we estimate it to be roughly comparable to 0.5 MAC, the ability of urethane to retard DA resembles that of enflurane. In Fig. 2, B the dose-effect curve is given. Since recovery from urethane anesthesia is very slow, we calculated the dose by considering the injections to be cumulative.

The widespread use of ethyl alcohol and therefore the possible practical consequences of an influence on DA made us include this agent in the test series. Results of two experiments are given in Fig. 3. In the first experiment 0.57 gram/kg, body weight (4 ml. of 40 volume per cent alcohol) was slowly injected I.V., followed after 70 minutes by a second injection of 0.72 gram/kg.
Fig. 2. A, Effect of the anesthetic urethane on the time constant of cone dark adaptation. Summarized results of one experiment. The arrows indicate the time at which an injection was given. Small numbers indicate additional dose and large numbers, cumulative dose. B, Dose of urethane plotted vs. time constant of dark adaptation. Different symbols indicate different experiments.

Fig. 3. A and B, Effect of ethyl alcohol on the rate of cone dark adaptation. Results from two experimental sessions. Arrows indicate when an injection was given. The alcohol was injected slowly, e.g., the large dose of 2.39 gram/kg, was infused during 40 min. The arrow was placed in the middle of this period. Small numbers indicate additional dose. A dose of 1 gram/kg, corresponds roughly with 0.1 per cent alcohol in the blood. C, Dose of alcohol vs. time constant of dark adaptation. The dose was calculated on the assumption that in monkeys as in man, alcohol is metabolized at the rate of 0.11 gram/kg, per hour.

(Fig. 3, A). Only after the second injection is any influence on the time constant of DA visible. The retarding effect on cone DA disappeared in 2 to 3 hours. In another experiment (Fig. 3, B) two injections, one of 0.97 gram/kg, and one of 2.3 gram/kg, were given. The consequence was a considerable retardation of DA. Five hours after the last injection the rate of DA was still slower than normal. After injection of the large alcohol dose the retina's sensitivity increased about 0.2 log unit. In Fig. 3, C the relation between the dose of alcohol and the resulting time constant of DA, as derived from the experiments of Fig. 3, A and B, is plotted. At alcohol levels where the 5 minute full bleach was followed by slow DA, a short full bleach was again found to be followed by a DA proceeding almost as fast as in a condition where no retarding agents were given.

Freon is the trade name for a score of very stable gasses and fluids which in their chemical structure resemble very closely the volatile anesthetics. To find out whether their action on DA also resembles that of the inhalation anesthetics, we chose one compound, Freon 11, which chemically resembles chloroform. At a concentration of
about 10 per cent of the inhalation mixture the physiological effects of Freon indeed closely resembled those of volatile anesthetics. Moreover, the DA was found to be retarded: the time constant increased from 60 to 200 seconds.

In the course of the experiments a few other substances that were for one reason or another available were tested for their influence on DA. In doses used for sedation the agents acetylazine (1 mg./kg.) and phencyclidine (0.6 mg./kg.) proved not to influence DA. Furthermore, ketamine at the anesthetic dose of 15 mg./kg. and all barbiturates tested (pentobarbital, methohexital, and thiopental) were also inactive in this respect.

Discussion. Although the experiments in this report were confined to one animal species, and describe only changes in cone DA, we feel justified in making the more general statement that certain anesthetics reduce the rate of photopigment regeneration in many (warm-blooded) vertebrates. Retinal densitometry showed that for halothane, the cause of retarded DA lies in a reduced rate of pigment regeneration for both rods and cones. An indication that rat DA might also be influenced by urethane can be found in the literature: Lewis with densitometry, and Dodt and Echle with the ERG found 90 per cent rod pigment regeneration to take 5 to 6 hours. Dowling, who used pentobarbital, found regeneration completed within 1.5 hours. In a densitometry study of Weale on guinea pigs, urethane was used in relatively high dosage (1.5 to 2 gram/kg.). This may explain his observation that the regeneration of the bleached pigment after exposure to a bright light proceeded at too small a rate to make feasible such [densitometry] measurements as those on the cat. Finally, Raskin and associates found a distinctly retarded recovery of the ERG b-wave after an injection of 5 gram/kg. of ethyl alcohol in rats.

It seems unlikely that metabolites play a role in the cause of retardation. First, the retarding action of volatile anesthetics appears within a few minutes after the onset of inhalation, whereas usually the formation of metabolites is a much slower process. Second, in view of the chemical stability of Freon it seems highly unlikely that this agent is metabolized at all. Third, ethyl alcohol has the property of being metabolized at a constant rate, i.e., independent of the alcohol concentration. In contrast, the retardation of pigment regeneration was found to be dependent on alcohol concentration.

We suggest two possible explanations for the mechanism of retarded pigment regeneration. First, it seems possible that anesthetics change the membrane of the outer segment or the pigment epithelium cell in such a way that the transport of the visual chromophore is slowed down. Expansion of the membrane, accompanied by a disordering of its components, is a common effect to all anesthetics. A second explanation could be that any of the enzyme systems involved in the restoration of 11-cis retinal is rendered less effective. According to the literature anesthetics can inhibit the physiological activities of enzymes.

Note that the formation of visual pigment from opsin and 11-cis retinal does not involve an enzyme system, which is in line with the observation that a fast DA after a short bleach, perhaps by means of a store of 11-cis retinal, is still possible.

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Key words: dark adaptation, anesthetics, cones, macaque monkey, ERG.

REFERENCES


The determination of the diffusion coefficient of krypton in rabbit ocular tissue. R. STRANG.

The validity of the inert gas clearance method for measuring choroidal blood flow has recently been demonstrated by studying the diffusion of krypton in ocular tissue. In this study the diffusion coefficients of krypton in rabbit ocular tissue were...