Influence of chlorpromazine on the rabbit electroretinogram

*J. M. Jagadeesh, H. C. Lee, and M. Salazar-Bookaman**

In anesthetized albino or nonalbino rabbits, a 3 to 8 mg/kg IV injection of chlorpromazine did not affect a-wave amplitude of the electroretinogram (ERG). However, immediately after the injection of the drug, b-wave amplitude increased. The maximum increase occurred between 35 to 50 min, and the recovery time varied between 5 to 8 hr. Initial changes in the b-wave amplitude to some extent were affected by systemic changes in the blood pressure. However, the b-wave amplitude remained high for a long time after the blood pressure reached preinjection value, indicating a local effect of the drug. There was no change in a- or b-wave latencies. Although in vitro a large quantity of chlorpromazine can be localized in the melanin granules from pigmented rabbit retina, in vivo the ERG b-wave changes caused by the small intravenous dose of the drug were similar in both albino and nonalbino rabbits.

Key words: chlorpromazine, rabbit, electroretinogram, melanin, binding

Many therapeutically useful drugs, including antipsychotic substances such as chlorpromazine, impair ocular function with or without apparent pathological changes.[1-6] Even though the literature reporting the visual side effects of chlorpromazine is very extensive, the information dealing with electrophysiological changes is rather limited.

Legros et al.[7] have studied the chronic effect of chlorpromazine in both albino and nonalbino rats. The animals were treated 6 days a week for 4 months with oral chlorpromazine at a dose of 20 mg/kg for the first 10 days, then 10 mg/kg for 3.5 months. It was observed that the b-wave amplitude in the electroretinogram (ERG) was reduced with respect to controls at the end of the fourth month and returned to normal value about 4 months after termination of drug treatment in albino rats. No statistically significant change in ERG in pigmented rats was observed. Biochemical estimation of chlorpromazine in the whole eye showed complete absence of the drug in the eye for albino rats. The quantity of the drug retained in the eye was dose-related for nonalbino rats.

On the other hand, Legros et al.,[8] in their experiments with dogs, have demonstrated that the amplitudes of a- and b-waves were diminished in both the treatment and post-treatment periods. They also found histological lesions in the eye. Chlorpromazine-induced ERG changes were irreversible. Since the early studies by Potts,[9] the visible retionotoxic effects of chlorpromazine that are observed in humans are equated with the drug binding by the uveal pigment. However, the studies in the albino and nonalbino rats appear to be paradoxical.[7] Thus the relationship between the drug binding by the pigment and its influence on the histopatho-

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logical as well as ERG changes has remained an unresolved issue. Hence, a comparative study in the albino and nonalbino rabbits was initiated to seek some explanation for the drug-induced alterations in the ERG from both types of animals.

Recording system

In the ERG recording system, light stimulus is provided by a Grass Model PS 22 photostimulator. The rabbit's head and the flash lamp are enclosed in a black-painted plastic globe which has a reflective inner surface (Drs. R. Jones and R. M. Hill, College of Optometry, The Ohio State University, Columbus, Ohio, personal communication, 1976). The globe, the lamp, and the rabbit's head are inside a large metal box, which serves to isolate the eye from possible external noise interference. The preamplifier and the rabbit's body are placed inside another metal box for the same reason.

The two metal boxes are connected to a common ground. The overall recording system has very low 60 Hz pickup, and the noise in the recorded signal is mainly of thermal origin. A Burian-Allen contact lens electrode was used to record ERGs. The ground lead was connected behind the ear. The signal was amplified by a Grass Model PS 16 amplifier. Capacitive coupling was used, and the amplifier bandwidth was set at 0.2 to 500 Hz. The gain was set at 1000. The signal was further amplified by a fixed gain of 5 by a homemade amplifier, Mono Op-7 of Precision Monolithic. The amplified ERG signal was connected to an ADAC Model 600-LSI-11 data-acquisition module consisting of a 64-channel multiplexer, a high-speed sample and hold, and a 12-bit analog-to-digital converter. The LSI 11/03 computer with a real time clock and 32K words of memory (Digital Equipment Corp.) was used to collect the ERG data. The LSI-11 has been linked to the PDP 11/34 computer installed in the main instrumentation room of the college. The PDP 11/34 is equipped with (1) VT 11 refresh graphics display, (2) RKO 5 cartridge disc, and (3) Tektronix interactive plotter. The ERG data was stored on the disc of the PDP 11/34 system and later analyzed with this large system.

Methods

The general anesthetics used were Ketalar (ketamine hydrochloride injection, NF), 100 mg/ml, manufactured by Parke, Davis & Co., Detroit, Mich., and Rompun (xylazine), 20 mg/ml injectable, manufactured by Haver-Lockhart Laboratories, Division of Bayvet Corp., Shawnee, Kan. The local anesthetic used was Alcaine (proparacaine hydrochloride, 0.5% ophthalmic solution) manufactured by Alcon Laboratories, Inc., Fort Worth, Texas. The ophthalmic solution used to dilate the pupil was 1% mydriacyl (tropicamide 1.0%) manufactured by Alcon Laboratories. Heparin solution was prepared from heparin sodium injection (porcine mucosal, 1000 USP U/ml), manufactured by Lypho-Med, Inc., Chicago, Ill.

The following solutions were prepared before each experiment: (1) 4 ml of ketamine + 4 ml of xylazine + 12 ml of 0.9% saline solution, injected IM into the rabbit to keep it anesthetized over the entire ERG experiment; (2) heparin solution prepared by diluting the 1000 USP U/ml solution to less than 20 USP U/ml with 0.9% saline solution.

A rabbit was removed from the animal room, weighed, and kept in the dark. After the above solutions were prepared and the syringes and the tubings filled with diluted heparin solution, all lights were turned off except for one localized light which was at a distance from where the rabbit was to be prepared for the experiment. The rabbit was then anesthetized by intramuscular injection of 50 mg/kg ketamine and 10 mg/kg xylazine. One needle was inserted into the ear vein of the rabbit, and another into a muscle near the leg. Both needles, with tubing and syringe connected, were taped to the animal and checked to be sure that they stayed securely in place. One or 2 drops each of 0.5% proparacaine hydrochloride and 1% tropicamide ophthalmic solutions were applied to the rabbit's eye. After this, the rabbit was put into the metal box, and the Burian-Allen bipolar electrode was placed on the corneal surface of the eye. The two leads were connected through coupling capacitors to the differential input connectors G1 and G2 of the Grass P16 preamplifier. A ground lead was connected to the rabbit's ear.

A few flashes of low-intensity light were then applied to produce several ERGs for examination on the cathode ray oscilloscope (CRO). After the electrode was adjusted properly and the ERGs were optimal as shown by the CRO trace, the metal box was closed, and the gap between the two metal boxes was sealed with black plastic covers. Airflow going into the metal box through a plastic tube was turned on and controlled at a suitable rate.

Light flashes were applied to the rabbit's eye every 1.5 to 2 min. The ERG amplitudes gradually approached a maximum value as the time the rabbit was kept in the dark progressed. In most cases, the rabbit ERG reached its maximum in

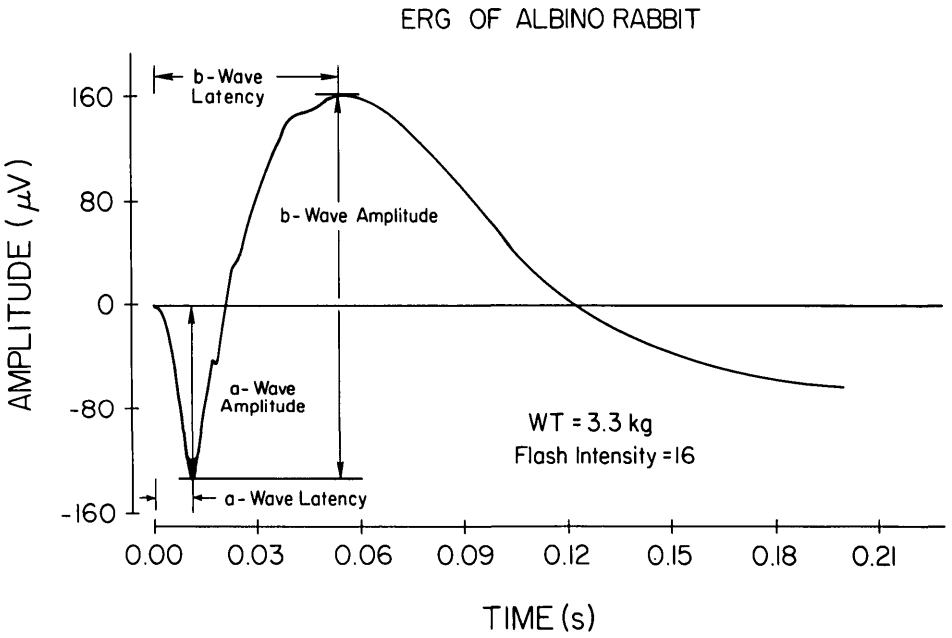

Fig. 1. Typical ERG plot for an anesthetized albino rabbit. Latency and amplitude parameters are indicated. The ERGs were digitized with a 12-bit analog-to-digital converter, and 4096 points were stored for each ERG with a sampling period of 0.5 msec.

about 10 to 15 min. The ERG amplitudes were monitored on the CRO. When they became constant, the chlorpromazine solution was slowly injected intravenously, and the ERGs were monitored and recorded on the computer disc. During the entire experiment, the rabbit was kept anesthetized through multidose administration of the diluted solution (ketamine + xylazine + saline solution) prepared earlier. About 10 mg/kg ketamine and 2 mg/kg xylazine were injected every 30 min. The dose and the interval depended on how deeply the rabbit was anesthetized and on the stability of the ERG signal. Usually, the change in ERG after drug injection was followed for 2 to 5 hr. The total duration of the experiment was nearly 6 hr. In some animals systemic blood pressure via carotid artery as well as ERG changes were monitored. The systolic and diastolic pressures were recorded on the physiograph (Narcobiosystem). The mean blood pressure was calculated as diastolic plus one-third the systolic pressure.

The analysis of the experimental data was via the computer programs in the PDP 11/34, and the results were plotted on a digital x-y plotter connected to the computer system. Preliminary experiments were conducted to establish proper procedures and appropriate instrument parameters before studies with chlorpromazine were made. Chlorpromazine was tested on 35 rabbits. Four rabbits died during the experiment. The ERG was very unstable, and there was considerable eye movement for six rabbits. Different rabbits were tested with doses ranging from 3 to 15 mg/kg.

In vitro localization of ^{3}H-chlorpromazine by the pigmented and albino rabbit retina. Albino and nonalbino rabbit retinas were isolated and homogenized in 2.5 ml of cold physiological salt solution in a glass Teflon homogenizer, with 8 to 10 strokes, at 600 rpm. The homogenate was incubated with ^{3}H-chlorpromazine, 244.2 nCi/ml (2.5×10^{-6}M), for 1 hr under constant oxygenation at 37° C. After the incubation period, the preparation was centrifuged at 43,500 ×g at 0° to 4° C, for 30 min. The pellet was rinsed twice with 5 ml each of 0.32M sucrose solution and was uniformly suspended in 4 ml of the isosmotic sucrose solution. The suspension was layered on top of discontinuous sucrose density-gradient tubes containing different gradients of sucrose. The gradient tubes were centrifuged in a swinging bucket (Beckman Rotor SW36) at 105,000 ×g for 1 hr in a Beckman Model L ultracentrifuge. The sucrose layers were carefully separated with Pasteur pipettes, and the volumes of the fractions internally

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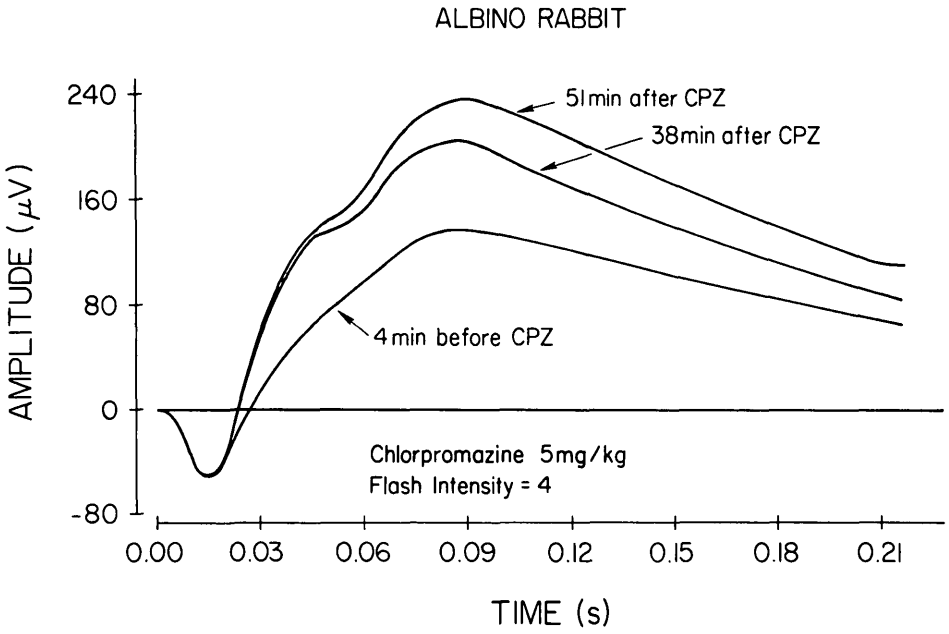

Fig. 2. Changes in a- and b-wave amplitudes as a function of time for albino rabbit before and after IV injection of chlorpromazine *(CPZ)*. The ERG waveshape was not affected by the injection of the drug other than the change in the b-wave amplitude. The results were similar for both albino and pigmented rabbits. Note that the a- and b-wave latencies have remained constant.

equalized with demineralized double-distilled water. Proteins were precipitated by adding 1 ml of 10% trichloroacetic acid solution to each fraction and removed by centrifuging in a desk model centrifuge for 10 min. The precipitate was subsequently washed twice with 0.5 ml of a 5% trichloroacetic acid solution. The supernatants were collected for determination of radioactivity by scintillation spectroscopy. Protein content of the tissue was determined in the precipitate by the method of Lowry et al.[10]

Results

From the experiments conducted on both albino and pigmented rabbits without injecting any drug, it was observed that (1) a period of 2 sec was sufficient to observe a- and b-waves, (2) a sampling rate of 2000 Hz was adequate to record the rapid oscillatory potentials, (3) the intensity levels I = 4,8,16 of the Grass PS 22 photostimulator were near the saturated stimulus levels of the b-wave, and (4) the amplitudes and latencies of both a- and b-waves practically remained constant over the entire period of the experiment with no drug administered to the rabbit. A typical ERG plot at an intensity level of I = 16 for an albino rabbit is shown in Fig. 1. This was an average of 2 ERGs. How the a- and b-wave peak amplitudes and latency periods were measured is indicated on this plot.

A series of ERGs taken before and after chlorpromazine was injected are shown in Fig. 2. There was a steady increase in the b-wave amplitude, and the a-wave amplitude was unaffected. This was a typical observation on all the rabbits studied, for both albino and pigmented rabbits. As is evident from the figure, there was no significant change in the ERG waveform after drug injection. A frequency spectrum analysis of the ERGs was performed with Fourier transform techniques. The frequency spectrum of the ERG waveforms before and after drug injection indicated no frequency shifts.

Soon after the drug was injected, the b-wave amplitude started increasing and reached a maximum value in 30 to 50 min, depending on the rabbit. The b-wave am-

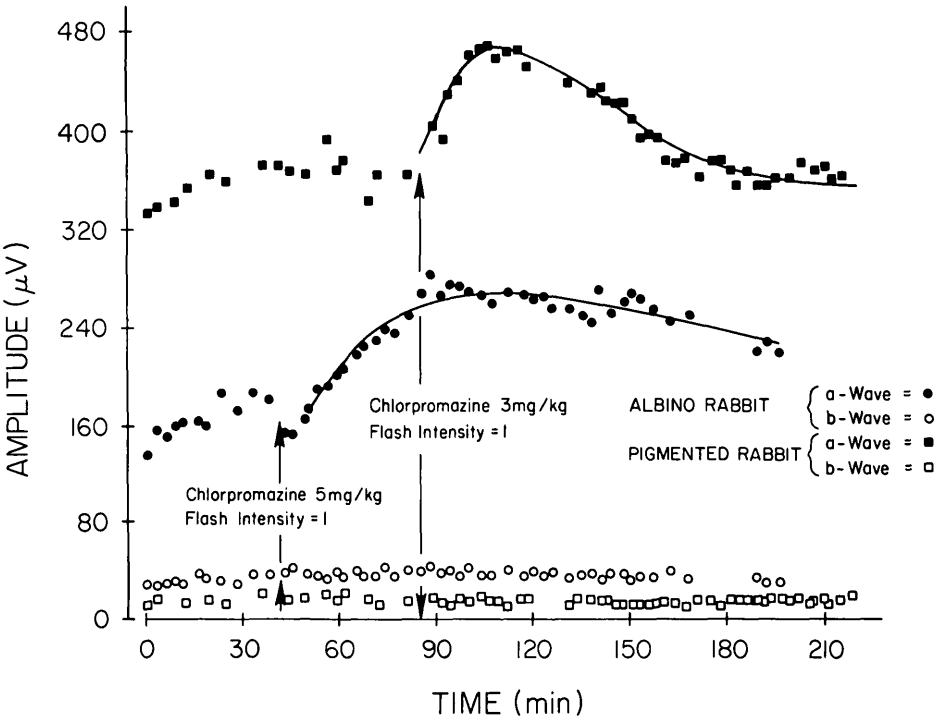

Fig. 3. Changes in a- and b-wave amplitudes as a function of time for albino and pigmented rabbits before and after intravenous injection of chlorpromazine. The increase in the b-wave amplitude was dose-related; higher doses induced greater increase. There were no statistically significant differences between the results obtained for pigmented and albino rabbits. Rate of rise, maximum increase, and the rate of fall of the b-wave amplitude varied from animal to animal.

plitude gradually decayed and reached the predrug values in about 4 to 6 hr for most of the rabbits. The rate of increase was faster than the rate of decrease. There was no change in this pattern for different light intensity levels. The latencies of both a- and b-waves prior to and after the injection of the drug were the same. There was no noticeable change in the a-wave amplitude. The results for both albino and pigmented rabbits were practically the same. Fig. 3 shows growth and decay of b-wave amplitude and constancy of a-wave amplitude as a function of time for both pigmented and albino rabbits.

A plot of a- and b-wave amplitude and the blood pressure is given in Fig. 4 for an albino rabbit. Within a few seconds after the drug was injected, the mean blood pressure dropped sharply and gradually increased. In most cases the blood pressure returned to the value before drug injection in 80 to 100 min after drug injection. The sharp fall in the mean blood pressure and a slight reduction in the b-wave amplitude were dose-related; higher doses induced higher reduction. With doses higher than 10 mg/kg, most often the animals did not recover. The mean blood pressure varied from animal to animal. The pattern was quite similar for both pigmented and albino rabbits. The b-wave amplitude remained above predrug level throughout the period of the experiment even though the blood pressure had returned to normal value.

Localization of ^{3}H-chlorpromazine in isolated rabbit retina by discontinuous sucrose density-gradient centrifugation. The distribution pattern of radioactivity in each type of retina was different (Fig. 5). In the albino rabbit retina, the greatest amount of ^{3}H-chlorpromazine was found in the lightest su-

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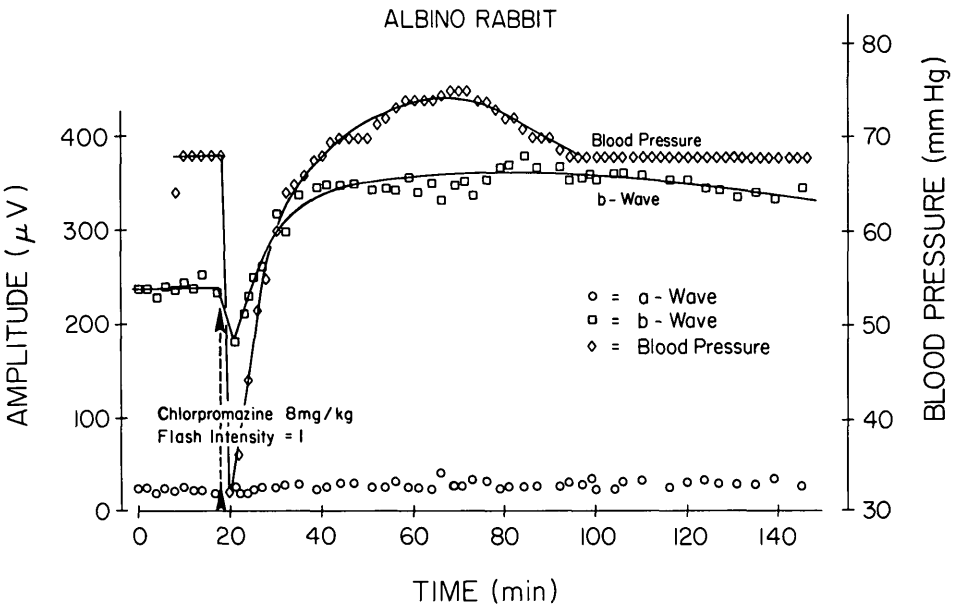

Fig. 4. Change in a- and b-wave amplitudes and arterial blood pressure after intravenous injection of chlorpromazine for albino rabbits. Note that the b-wave amplitude remained higher than preinjection level for a long time after the blood pressure had reached preinjection level. Results were similar for pigmented rabbits.

crose layers, 0.32M and 1.0M. Approximately 43% of the total bound drug remained in the top gradients, where 31% separated in the 1.0M sucrose layer. In the pigmented rabbit retina, the largest amount of drug (41% of the total bound drug) was found in the sediment of melanin granules at the bottom of the gradient. It was also found that the amount of ^{3}H-chlorpromazine bound in the 2.0M sucrose fraction (16% of the total drug bound) obtained from the pigmented tissue was significantly higher ($p < 0.05$) in the retinas of both albino and nonalbino rabbits, except in the granules sediment which contained the largest amount of protein.

Discussion

In this study, chlorpromazine caused a gradual increase in b-wave amplitude. The drug did not affect either a-wave amplitude or a- and b-wave latencies. Although in vitro a large quantity of chlorpromazine can be localized in the melanin granules, in vivo the ERG changes by the drug were similar in albino as well as pigmented rabbits. Thus initial ERG changes in animals after chlorpromazine injection are independent of pigmentation.

Electrophysiological studies in cats[11] and sheep[12] have shown reduction in the b-wave of the ERG following administration of chlorpromazine. However, our experiments on rabbits have demonstrated that the b-wave amplitude increased after intravenous injection of the drug. Although in each species different doses were used, it seems unlikely that dosage alone can explain the reversal seen in present experiments in rabbits. Hence, the difference in the ERG responses seen gives rise to an interesting question concerning possible variation in the mechanisms of drug action in the several animal species.

The greatest difference in morphology between species is in the relative number of specific types of cells and in the synaptic structures.[13] Two major differences among cats and sheep in comparison with rabbits are the retina–optic nerve vascular system and the contents of neurotransmitters within the retina. The retinal vascular systems in cats, rats, sheep, and man belong to the so-called

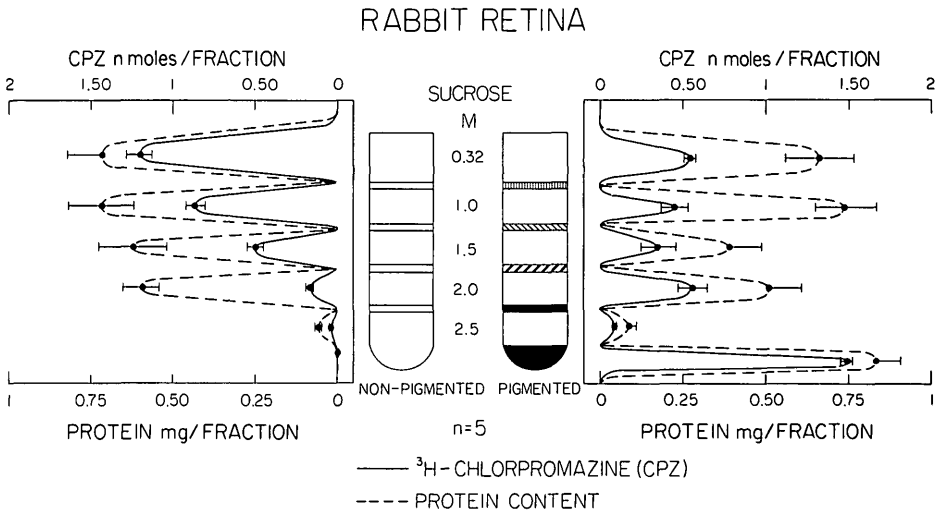

Fig. 5. In vitro localization of ^{3}H-chlorpromazine in pigmented and nonpigmented rabbit retina by discontinuous sucrose density-gradient centrifugation. Each point represents the mean of five observations ± S.E.M. ^{3}H-chlorpromazine bound is expressed as nanomoles per fraction.

holangiotic type, which means the retina is completely vascularized with a special capillary structure of a very dense network. On the other hand, the retinal vascular system in the rabbit is considered an anangiotic type, which means almost complete absence of blood vessels in the retina. The relevance of anatomical differences in terms of ERG changes remain to be clarified.

Neimeyer[14] has conducted an exhaustive study on the relationship between the ERG and the variations of biophysical factors such as flow rate and oxygenation in isolated perfused eyes. He has demonstrated that the b-wave amplitude increased when the relative flow rate was increased. Implications of these findings could not be directly translated to our experimental results. It is rather impractical to measure exact vascular blood flow into the rabbits eye in an in vivo experiment. However, the arterial blood pressure can be used as a reasonable indicator of blood circulation. There seems to be some correlation between the blood pressure changes and the b-wave amplitude, as indicated by a sharp decrease in blood pressure followed by a small fall in the b-wave amplitude soon after the drug was injected. It is also likely that the overshoot in the blood pressure has an effect on the increased b-wave amplitude. However, since the b-wave amplitude remained high even after the blood pressure has reached a normal value, chlorpromazine must have some local effect.

Rabbit retina contains more dopamine than does rat, frog, guinea pig, or toad retina.[15] Therefore drugs which have a specific action on some particular neural transmitter are likely to show varying effects in animal species having different transmitters. Chlorpromazine has been shown to react with or block the synaptic receptors[16] and hence should be capable of producing varying effects in different types of retinas. If the concept that dopamine is inhibitory is accepted, blockage of its effects by chlorpromazine should result in an overall stimulatory effect in the retinas of animals mediated by dopamine. Moreover, drug metabolism in different animal species is likely to be different, and the way the ERG is affected may depend upon these metabolic differences rather than upon the structural differences or transmitters of the retina.

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