ELECTRON MICROSCOPE AND OPTICAL DIFFRACTION STUDIES ON INTERACTION OF PSYCOACTIVE DRUGS WITH BIOMEMBRANES

R. S. KHARE AND R. K. MISHRA

Department of Biophysics, All-India Institute of Medical Sciences, New Delhi-110016

ABSTRACT

Membrane-drug interactions have been studied by optical diffraction from electron micrographs. Drug induced changes in the membrane association accompanied by changes in the optical diffraction patterns were observed.

INTRODUCTION

THE current interest in the structure of biomembranes[1,2] has stimulated interest in membrane-drug interactions. Most of the studies relating to the interaction of psycoactive and other pharmacologically and physiologically active compounds with biomembranes and with various lipid model systems have been confined to the surface activity of the drug molecules, metabolic turnover and electrical activity of membranes[3-6]. Electron micrographs of tissue sections and of isolated membrane fragments have emphasized various lamellar and globular or other subunits aspects of structure and provided approximate dimensions for a wide variety of biomembranes[7]. Structural and functional modifications induced in muscle microsomes by trypsin[8] and interaction of polyene antibiotic filipin and derivatives with erythrocyte membrane and lipid aqueous dispersions[9] *in vitro* excitation of purified membrane fragments by cholinergic agonists[10] and the effect of acetylcholine in lipid proteolipid membranes[11] have been studied with electron microscopy. Recently using autoradiography in the electron microscope Porter *et al.*[12] have specified the sites and membrane density of acetylcholine receptors at single endplate.

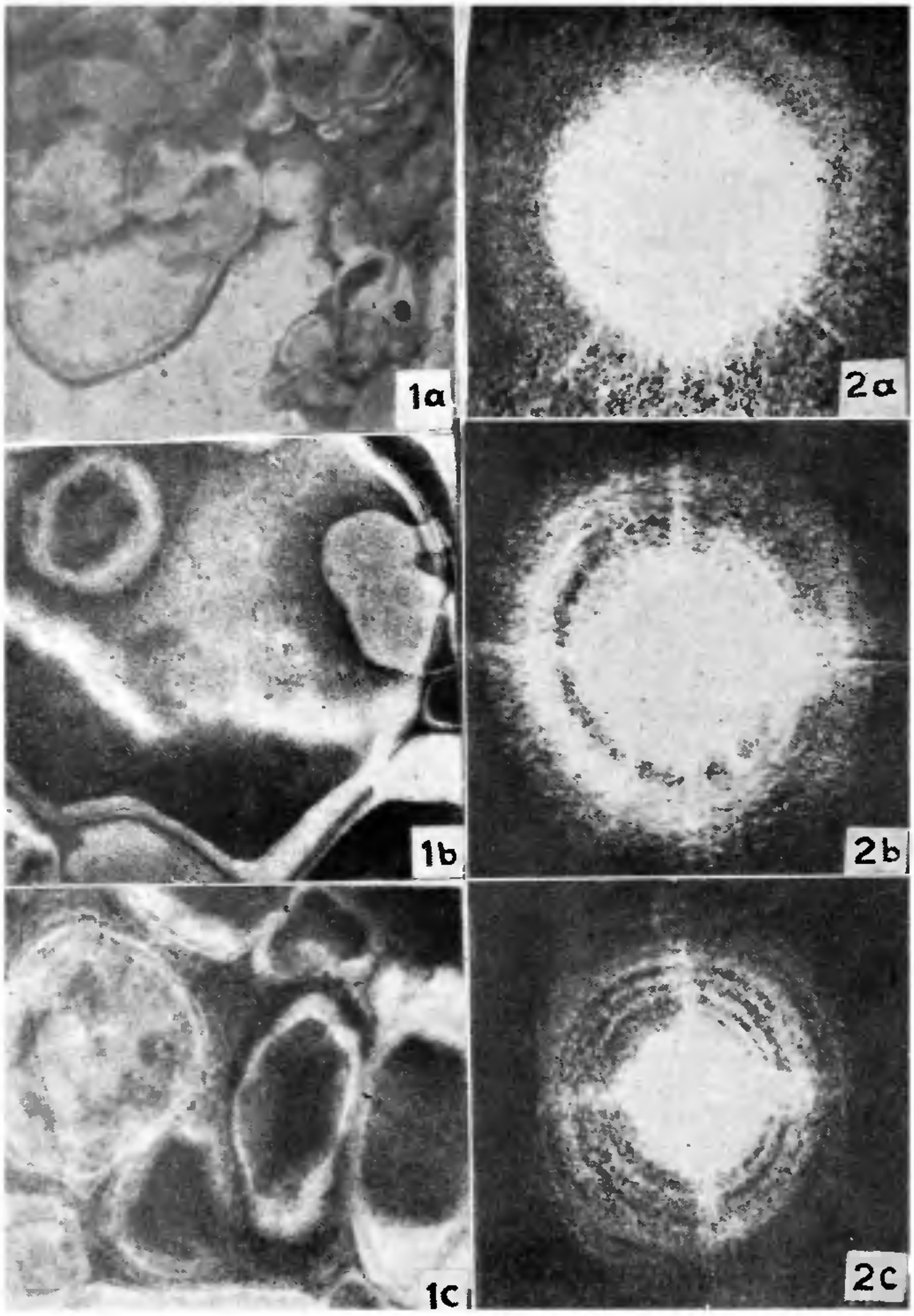
The analysis of electron micrographs by optical diffraction (structural spectrography) provides a useful method for the detection of periodic spacings[13,14]. Lipid-water phases[15,16], ultrastructure of cell wall[17,18], retinal receptor disc membrane[19], cytochrome oxidase membranes[20], and purple membrane[21] have been studied by optical diffraction analysis of electron micrographs of negatively stained or freeze-etched specimens. By a similar analysis Gilula *et al.*[22] showed that the membrane particles and alternate vertices of the intercellular spetal sheet are congruent and therefore superposable. Recently, we have reported evidence of structural changes in neuronal membranes under the action of drugs using interference microscopy[23] and electron diffraction[24]. The preliminary findings reported here concern the application of electron microscopy of negatively stained neuronal membrane fragments, and the interaction of some chosen neurotropic drugs on these membranes, with particular reference to the ultrastructural features and their analysis by optical diffraction.

MATERIALS AND METHOD

The neuronal membranes from rat's brain were isolated by a procedure based essentially on Neville's method and described earlier[23]. The neurotropic drugs used were acetylcholine chloride, atropine sulphate, caffeine and sodium barbital, their amount used being in ratio close to their physiological dose. Micrographs were obtained with Hitachi HU 11A electron microscope using 50 KV accelerating voltage, 200 μm condenser and 50 μm objective apertures. Magnifications were calibrated with a grating replica (2160 l/mm). Membrane preparations were negatively stained with phosphotunstic acid by the droplet technique. A droplet of membrane suspension was placed on a carbon-collodion covered copper grid, allowed to remain for a few seconds and then rinsed off with staining solution, and the grid blotted dry. The optical diffractometer used in these studies was of a horizontal type[25], however, one lens system was used after a design of Arvindakshan[26]. The light source was a Spectra Physics He-Ne laser model 120, transform lens was 2 m focal length. The optical path was folded by using a reflecting mirror. The mirror did not introduce any measurable distortion. In addition to reducing the overall size of the instrument, this has the advantage that the micrograph, and the ground-glass viewing screen are all within easy reach of the operator. For some experiments optical microscope (Zeiss) modified after Gall[27] was also used as a diffractometer.

The electron image plates or contact copies or reduced copies made from them were used for the optical diffraction without oil immersion[28], micrograph magnifications being in the range of 50,000 to 100,000. Areas selected for diffraction were masked with opaque adhesive tape applied to non-emulsion side of the glass plate. Diffraction patterns were recorded on Kodak High Contrast film, developed in Kodak D 19. The optical diffraction pattern negatives were measured on a

projectroscope. Measurement of the radial co-ordinate of each principal maximum was somewhat difficult, since the central peak intensity of the reflection was not clearly defined. The radial co-ordinate of a reflection was half the distance measured across the equivalent reflections. About 10 diffraction patterns were measured. They were selected from a somewhat larger number of patterns. The diffraction patterns were calibrated by comparison with patterns obtained from a bar grid. The diffractometer constant,



FIGS. 1–2. Fig. 1. Electron micrographs of negatively stained membrane preparations. (*a*) Normal neuronal membrane, × 60,000; (*b*) Membranes treated with atropine sulphate, × 75,000; (*c*) Membranes treated with acetylcholine *plus* atropine sulphate, × 75,000. Fig. 2. Optical diffraction patterns obtained from electron micrographs. (*a*) Normal membranes; (*b*) Acetylcholine treated membranes; (*c*) Caffeine treated membranes.

10 diffraction patterns were measured. They were

d = real space distance × reciprocal space distance

was equal to 0·077 mm². Photographic reproduction of diffraction patterns posed considerable

difficulty due to nonuniform and poor background. Special methods for improved reproduction of diffraction patterns and electron micrographs[29-31] have been described, however, these techniques have not been used in the present work.

Results and Discussion

Some of the electron micrographs and optical diffraction patterns are shown in Figs. 1 and 2 respectively. Normal membranes appear as smooth continuous structures. On treatment with acetylcholine the membranes show areas of rarefraction and condensation of particles, which appear to be micelles, composing of membranes. Atropine sulphate which antagonises the action of acetylcholine at most of the sites produced fragmentation of membranes which was checked by combination with acetylcholine as evident from micrograph of membranes treated both with acetylcholine *plus* atropine. Appearance of membranes treated by barbitone and caffeine was considerably different from all other cases. The precise interpretation of the structures, observed in negatively stained preparations of neuronal membranes and their altered aggregation in the presence of neurotropic drugs, is problematic at present. Computer anaylsis and image reconstruction[32] would be desirable for a quantitative interpretation. Some of the qualitative inferences one may draw from these observations are : State of membrane fragment aggregation is altered by acetylcholine. The enhanced aggregation of micelles may open pores filled with water, thus facilitating diffusion across membranes and altered polarization. In general, the state of membrane organization can be modulated and can be rendered dynamically transformable by the presence of very small amount of the drugs used in these experiments.

Optical analysis of electron micrographs provides information about the nature of the order in the membrane aggregates. It shows that extensive regular arrays of the membrane subunits are not present, under the present experimental conditions of isolation and fixation, and that the order is limited to the nearest-neighbour. The commonest repeat distances observed in these studies have been summarized in Table I. Other repeat distances were approximately higher orders of these primary distances, suggesting overall random lammelar distribution of membrane fragments. Although no general conclusions can be drawn with this limited data, these studies also suggest that neurotropic drugs induce changes in the membrane association accompanied by intramolecular variation in the conformation of membrane subunits. The results obtained are in qualitative agreement with our studies on membrane-drug interaction by interference

Table I

Structural spectrographic data

Specimen	Primary repeat distance nm
Normal membrane ..	10·5±1·0
Normal membrane + Acetylcholine ..	8·5±0·5
Normal membrane + Atropine sulphate ..	8·2±0·5
Normal membrane + Caffeine ..	9·5±0·4
Normal membrane + Barbitone ..	8·1±0·5

microscopy[23], electron diffraction[24], circular dichroism and X-ray diffraction (being published elsewhere). Briefly, reasons for choosing various drugs used in the present study are as follows: Acetylcholine is a well-known neurotransmitter, atropine is best known cholinergic antagonist and also possesses some anticonvulsant and mild local anesthetic properties. Caffeine is known to be a powerful stimulant of the central nervous system at all levels. The barbiturates are hypnotic drugs, used for the induction of sleep, and they have a depressant effect upon the CNS.

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THE GRAAFIAN FOLLICLE IN SOME INDIAN BATS

A. GOPALAKRISHNA, A. MADHAVAN, R. S. THAKUR AND (MISS) GOPA RAJGOPAL
Department of Zoology, Institute of Science, Nagpur

THE enormous hypertrophy of the cells of the discus proligerus (cumulus oophorus) with the near complete obliteration of the antrum of the Graafian follicle has been noticed in the ovaries of some vespertilionid bats inhabiting cold and temperate regions (Wimsatt, 1944; Sluiter and Bels, 1951; Pearson *et al.*, 1952; Wimsatt and Kallen, 1957). In all these cases the Graafian follicle remains in an almost unaltered condition during the winter months when the female undergoes hibernation after coming to oestrus during autumn. Wimsatt and Kallen (1957) noticed that the hypertrophied cells of the discus proligerus of such follicles contain abundant quantities of glycogen, and considered that these modification are "an adaptation to meet the energy requirements of the ovum-follicle complex over the prolonged period of dormancy, during which time the metabolism of the animal is drastically reduced". However, the Graafian follicles of the British rhinolophid bats do not exhibit such histological peculiarities, and the cells of the discus proligerus do not hypertrophy, although these bats also undergo a long period of post-copulatory hibernation throughout winter when the Graafian follicle with a large antrum remains almost unchanged in the ovary (Matthews, 1937).

Since the structure of the Graafian follicle varies so much even amongst the hibernating bats inhabiting cold climates, it was felt that it would be interesting to make a comparative study of the Graafian follicles of some tropical bats belonging to different families. The present report embodies the description of the Graafian follicle of *Pteropus giganteus giganteus* (Pteropidae), *Megaderma lyra lyra* (Megadermatidae), *Rhinolophus rouxi* (Rhinolophidae), *Hipposideros speoris* (Hipposideridae), *Pipistrellus ceylonicus chrysothrix*, *P. mimus mimus* and *P. dormeri* (all belonging to Vespertilionidae). None of these species undergoes hibernation as noticed in the bats of cold and temperate regions.

Figures 1–7 illustrate the structure of the fully developed Graafian follicle in the seven species of bats studied here. Whereas the Graafian follicle of *Pteropus*, *Megaderma*, *Rhinolophus* and *Hipposideros* presents a picture typical of the Graafian follicle of most mammals in possessing a large antral cavity and in having the ovum surrounded by one or two layers of small cumulus cells (Figs. 1–4), the mature follicles of the vespertilionid species (Figs. 5–7) studied here have a different structure, and are nearly similar to those of some of the hibernating vespertilionids inhabiting cold climates. In all the vespertilionids studied here the cells of the discus proligerus undergo enormous hypertrophy, and cellular bridges extend from the enlarged discus to the granulosa layers resulting in the reduction of the antral cavity to one or a few small spaces. Further, the hypertrophied cells of the discus proligerus contain numerous fluid-filled vacuoles (Fig. 9). The peculiar appearance of the mature follicle of the vespertilionid bats is, at least, partly due to the accumulation of secretions within the cumulus cells themselves. This is evident from the fact that the follicle cells are small and compactly arranged at the multilaminar and early vesicular stages of development of the follicle. Hence,