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INTERMEMBRANE LINKAGE MEDIATED BY TUBULIN

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Two membranes from brain lipids were formed in the presence of brain tubulin and their electrical potentials were simultaneously measured. When electrical pulses were applied across one of them, displacements of the potential of the other membrane were found even when the membranes were not in contact. This effect was observed only in the presence of polymerized tubulin. It was not found in the presence of depolymerized tubulin or in other control experiments. The findings suggest that the microtubule fiber networks may serve as an interconnecting system between membranes or membrane bounded compartments.

The microtubules consisting of tubulin subunits are ubiquitous cytoskeletal elements, which are involved in a variety of cellular processes such as maintenance of cell shape, cell motion, synaptic events, and excitability (1-5). Many of these processes depend on the coupling between events occurring in different membranes or membrane regions accompanied by defined changes of their mechanical and electrical properties. Recently the functioning of the cytoskeleton as transducer of signal between plasma membrane and nucleus has been considered (6). It is possible to suggest that the microtubules may interconnect and transmit signals between defined membranes or cellular compartments, thus playing a role in the structure-functional organization of the cells (7). Several studies showed the presence of membrane bound tubulin (8-10) and the tight associations of microtubules with membranes (11-13). Periodically located close contacts between microtubules and defined membrane regions (2,13) or membrane vesicles (12) are frequently observed, but the linking role of the microtubules has not been examined. Here we have investigated this eventual function of the microtubules by using a technique for formation of two bilayer lipid membranes (BLMs). The method of bilayer

Abbreviations: BLM - bilayer lipid membrane; MES - 2-(N-morpholino) ethanesulfonic acid; EGTA - ethyleneglycol-bis (2-amnoethyl ether) N,N,N'N'-tetracetic acid; GTP - guanosine 5'-triphosphate.

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lipid membrane (BLM) has been successfully used for investigating the interactions between membranes and different cellular components (14,15). We employed it also for examining the membrane-cytoskeleton relationships (16). An adequate method for studying the functions of such complex systems should be a double- membrane technique as described here.

EXPERIMENTAL PART

The membrane forming solution for BLMs contained brain lipids isolated (17) from the pellet obtained after the first ultracentrifugation step during the preparation procedure of tubulin, the latter being performed according to Shelanski et al. (18) by two cycles of polymerization and depolymerization. The membranes were formed from brain lipids (2%) in n-octane on the holes of the teflon chamber shown in Fig. 1. by the standard method (14). The bathing solution contained 0.1 M MES (pH 6.5), 10^{-3} M EGTA and 5 x 10^{-4} M $MgCl_2$ (MES-buffer). GTP (10^{-3} M) was added for tubulin polymerization. The whole volume of the teflon cell consisting of three compartments is 1.6 ml. The diameter of the holes is 2.2 mm. The formation of the bilayer membranes could be observed under the microscope from every side of the chamber. Dark field illumination was used. The initial distance between the membranes was 0.8 mm. They were bulged out and apposed against each other by creating a negative hydrostatic pressure in the intermediate compartment 3 when the level of the solution in this compartment was lowered gradually by a microsyringe. A narrow slit of 0.1 mm between the two walls of the intermediate compartment was located 2 mm above the upper rims of the two holes for membrane formation. It was calibrated during the construction of the chamber and served as a marker for fixing the intermembrane distance of 0.1 mm. The dark field illumination used permitted a clear visualization of the membranes during their inflation and their blackening. After the formation of the membranes, rectangular electrical pulses were passed between the first and the third compartment (Fig. 1) and the resulting displacements of the potentials across the two membranes were measured simultaneously by using the electrodes 5 and 6. The appearance of potential changes across the second membrane are interpreted in terms of

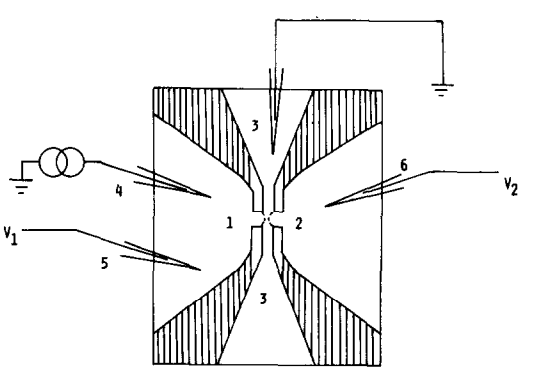

Fig. 1. Schematic representation of the set up used for investigating two membranes situated against each other. The teflon cell consisted of three compartments 1, 2, 3. Electrical pulses were generated by a stimulator and were passed through the electrode 4 between the compartment 1 and the grounded compartment 3. The resulting displacements in the potentials across the first and across the second membranes were measured by using Ag/AgCl electrodes 5 and 6, respectively, connected to two electrometers. The electrical signals were displayed on a chart recorder.

electrical coupling between the membranes. The BLMs in this chamber were situated facing each other and the microtubules could eventually interconnect them only across the intermediate compartment (labelled #3 in Fig. 1).

The tubulin polymerization was performed in the chamber 1 h before the formation of the membranes in the presence of GTP at final concentration of 1 mM added from a stock solution of MES-buffer. Relatively high protein concentration (1.5 mg/ml) was used in order to ensure the polymerization process. At this protein concentration (determined according to Lowry et al. (19)) we could not obtain stable membranes under asymmetrical addition of tubulin on one side of the BLMs. Therefore, depolymerized or polymerized form of tubulin was present in all the compartments of the chamber. GTP was not added to the solution when the effect of depolymerized tubulin was investigated. Four experiments for each case were performed, showing similar results.

RESULTS AND DISCUSSION

When the two BLMs were not in physical contact no electrical coupling between the two membranes was observed in the presence of depolymerized tubulin. A change in V_2 occurred after a close contact was established between the membranes (Fig. 2, thick arrow). The potential displacement in the second

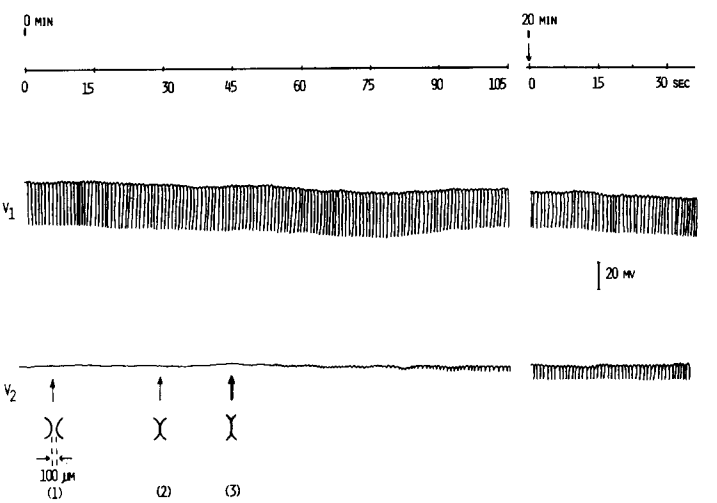

Fig. 2. Interrelationships between the two membranes in the presence of depolymerized tubulin (no added GTP). The membranes were formed on the two holes of the chamber shown in Fig. 1. Electric pulses (5×10^{-8} A, 30 ms, 1.3 Hz) were applied across the first membrane and the resulting changes of the potentials of this (V_1), as well as of the second (V_2) membrane were recorded. The arrows below the records indicate the different steps during the apposition of the two membranes against each other: (1) when the distance between the membranes was fixed at 0.1 mm, (2) when they were brought into contact, (3) close contact between membrane areas. After the transition from stage 2 to stage 3, the mutual disposition of the membranes during their interaction remained unchanged. After the interruption, the time calibration in seconds began from 0. The arrow above the time scale shows that after the interruption the record began at the 20-th minute as indicated.

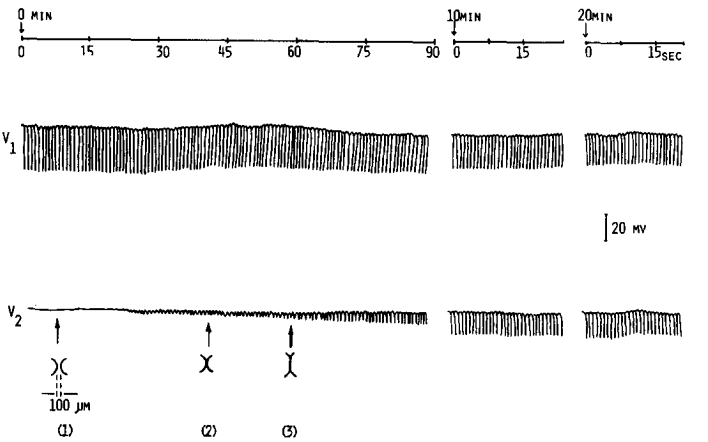

Fig. 3. Interrelationships between the two membranes in the presence of polymerized tubulin (added GTP at 1 mM final concentration). After every interruption the time calibration in seconds begins from 0. The first and the second arrows above the time scale indicate that after the first and the second interruptions the records began at the 10-th and the 20-th minute, respectively. The other conditions and the designations are the same as given in Fig. 2.

membrane gradually increased after this moment (Arrow 3, Fig. 2), reaching plateau values 20-25 minutes after the apposition of the membranes.

In the presence of polymerized tubulin (Fig. 3) electrical coupling was observed even when the intermembrane distance was 0.1 mm. After physically contacting the membranes, the amplitude of the potential changes in the second membrane increased faster, reaching plateau values in 15-20 minutes. These changes were more pronounced than in the presence of depolymerized tubulin shown in Fig. 2. In the presence of the latter the potential displacements induced in the second membrane were approximately two times lower than in the presence of polymerized tubulin 20 minutes after the apposition of the membranes. The findings can be interpreted in favor of intermembrane linkage mediated by polymerized tubulin. However, not only the typical microtubule fibers, but also some amorphous proteinaceous aggregates could be involved in this event. The specificity of the effect was tested by investigating the influence of colchicine, which is a tubulin polymerization-inhibiting agent, and of another protein, albumin, on the electrical coupling process. In three experiments (data not shown), in the presence of colchicine (5×10^{-4} M) added before the tubulin polymerization or of albumin instead of tubulin in the same

buffer and at the same concentration (1.5 mg/ml), no electrical coupling was found when the membranes were not in contact (at 0.1 mm intermembrane distance). It was important to test if colchicine may exert some direct effect on the electrical properties of membranes. Colchicine was found to influence the electrical events on the axonal membrane at much higher concentration (10^{-2} M) (5) than that used in this study, but its effect was thought to be due only to this tubulin polymerization inhibiting activity and not to unspecific perturbations of the membrane structure. In 6 experiments we did not find any effect of colchicine at $5\text{-}10^{-4}$ M on the membrane resistance and potential and on the intermembrane coupling in the absence of tubulin. In additional experiments a colchicine analog lumicolchicine which does not inhibit the tubulin polymerization was not found to influence the tubulin-induced intermembrane coupling and the same effect as that shown in Fig. 3 was observed in this case.

The presence of GTP was the single difference in the conditions when the influence of polymarized tubulin was compared with that of depolymerized tubulin. Therefore, it was needed to verify what should be the effect of GTP on the membrane electrical properties. No influence of 1 mM GTP on the resistance and potential of BLMs as well as on the intermembrane coupling in the absence of tubulin was found.

Thus, it seems reasonable to conclude that the observed effect is due to the interconnective function of microtubules between the two membranes. At present, it is difficult to suggest a detailed underlying mechanism owing to the lack of information about the electrical properties of the microtubular network systems as a whole and of the microtubule wall. It should be noted that its thickness, 4-5 nm, is comparable to that of the bilayer membranes. If its resistance should be high and if the cylindrical microtubules may interconnect the two membranes across the intermediate compartments, a mechanism based on the ionic conduction through the microtubule lumen can be suggested. Although the length of the microtubules may reach several hundred microns, this might not be the single explanation of the findings. Another mechanism may

involve electrically-induced polarization and conformational changes of microtubule components. The very high charge asymmetry of the tubulin molecules (20), as well as the previous findings showing microtubule orientation in low intensity electric fields (7) are suggestive in this direction. Other explanations are also possible, but whatever the concrete mechanisms of the observed effects, they may be related to the eventual communicative function of the microtubular system.

The intermembrane linking role of the tubulin fiber networks may be of particular importance for the integration and coordination of the processes occurring in different cellular compartments. For example, they may be tightly involved in the integrative function of the neuron. The latter can receive hundreds of input signals from the synaptic endings of the neurites of neighboring cells, but every neuron produces only one output signal. The mechanism of this integrative function of the neuron is not clear. It is possible to speculate that some well organized cytoplasmic structures such as the microtubules and some other cytoskeletal fibers can transfer the information in the form of electrical signals and conformational changes from the cellular membrane to the nucleus, where at least one of the three types of cell memory, the long-term memory, can be located. The same structures may be responsible for the transfer of information from the nucleus back to the cellular membrane, where in defined region the output signal of the neuron in form of action potential is generated.

For further detailed studies in this direction it should be needed to use more complex double membrane systems reconstituting different subcellular membranes and to examine the intermembrane linking function of various cytoskeletal components.

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