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INTERACTION BETWEEN ISOLATED RAT BRAIN SYNAPTIC VESICLES AND PLANAR BILAYER MEMBRANES

L. R. Tinyakova, V. A. Tverdislov, L. V. Yakovenko, and R. N. Glebov

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The hypothesis is now generally accepted that mediator secretion takes place by exocytosis, which consists of several stages: 1) coupling of depolarization and secretion of mediators and intracellular accumulation of Ca^{++} ions, 2) Ca-dependent transport of synaptic vesicles (SV) to the active zone of synapses, with the participation of cytoskeletal contractile proteins, 3) Ca-dependent joining of SV with complementary specialized sites on the inner surface of presynaptic membranes (pre-SM), Ca-dependent approximation and fusion of SV with pre-SM, i.e., intermembranous interaction [2]. Interaction of SV with pre-SM may take place through protein structures [3]. However, models of this interaction based only on the properties of phospholipids exist [8]. In the study of exocytosis of mediators it is interesting to investigate systems which model it *in vitro* and, in particular, systems of isolated SV-artificial bilayer lipid membranes (BLM). This system resembles most closely the liposomes-BLM system in which many aspects of intermembranous interactions were studied previously [5, 6, 11, 12]. Methods of studying interaction of BML with liposomes [4] and with lipoprotein complexes [7] have now been developed for systems including BLM. These methods are based on analysis of changes in some electrical characteristics of BLM, induced by the interactions being studied, and determined by changes in parameters of BLM such as capacity, conductivity, the intermembrane potential jump, and the modulus of elasticity.

In the investigation described below changes in conductivity of laminar BLM were measured during their interaction with isolated SV from whole rat brain. By this method it is possible to work with native SV, by which it compares favorably with that described in [10], in which SV filled with the fluorescent dye calcein were obtained, as a result of which their volume was increased by 50-100 times. Development of the model of mediator exocytosis, not yet described in the literature, may be useful for the study of secretory processes both under normal conditions and when disturbed as a result of various forms of pathology of the nervous system.

EXPERIMENTAL METHOD

The SV fraction was isolated from whole brain of rats weighing 150-200 g [1]. For this purpose, after removal of the nuclei from a 20% brain homogenate (0.32 M sucrose, 20 mM Tris-HCl, pH 7.4, 0.1 mM EDTA), unpurified synaptosomes were obtained (10,000g, 10 min), and were subjected to osmotic shock by suspending the residue in distilled water (at the rate of 4 ml of water to residue obtained from 1 g brain tissue). After freezing at −20°C and thawing the suspension was incubated in 1 mM EDTA solution for 10 min, then centrifuged for 30 min

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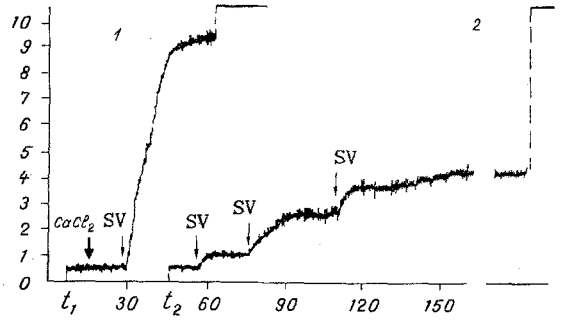

Fig. 1. Kinetics of change in current through membrane. 1) On addition of SV in the presence of 1 mM $CaCl_2$; 2) without $CaCl_2$. Arrows indicate times of addition of 10 µl SV up to a final concentration of 4 µg protein/ml. Abscissa, time (in min); ordinate, current (in conventional units — c.u.). t_1 and t_2) Time at which membranes were formed. 1 c.u. = 2.2 pA. Continuous line corresponds to level of current through pore after destruction of membranes, broken lines — time of destruction of BLM. Typical curves are shown.

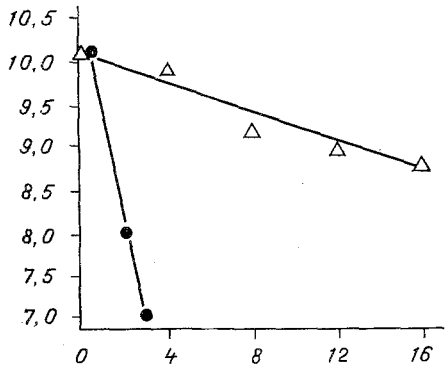

Fig. 2. Dependence of conductivity on SV concentration. Abscissa, protein concentration in SV (µg/ml); ordinate, —log G (G in Ω). Triangles — without $CaCl_2$, filled circles — in presence of 1 mM $CaCl_2$. Averaged data from three or four experiments.

at 18,000g, after which the supernatant was centrifuged for 30 min at 55,000g. The resulting supernatant was centrifuged for 40 min at 120,000g. The residue was the SV fraction. The fraction was suspended in 0.25 M sucrose containing 20 mM Tris-HCl, pH 7.4, and kept at —10°C. The preparation used had been thawed once. The protein concentration was determined by Lowry's method.

BLM were formed by the method of Miller at al. [9] on a pore 0.7 mm in diameter in the wall of a Teflon cuvette, containing a solution of 0.1 M KCl, 20 mM Tris-HCl, pH 7.4. To form BLM a mixture of azolectin and phosphatidylserine (PS) or dioleylphosphatidylcholine (DPC) in the ratio of 9:1, dissolved in n-decane in a concentration of 40 mg/ml was used. In the course of the experiments a constant voltage (E = 15 mV) was applied to the BLM. The voltage drop (V) on a standard resistance (R_0 = 680 MΩ), connected in series with the membrane, was measured by means of an LPU-01 electrometric amplifier. Membrane conductivity was expressed by the formula

$$G = \frac{V}{R_0 (E - V)}.$$

EXPERIMENTAL RESULTS

On addition of SV (3-25 μg protein/ml) to the aqueous phase on one side of a BLM formed from azolectin and DPC, conductivity was unchanged in the absence of Ca^{++}. This is in agreement with data in the literature [5, 6], indicating that an essential condition for fusion is the presence of acid (negatively charged) phospholipids in the composition of the BLM and the presence of Ca^{++} ions in millimolar concentrations.

On the addition of SV in a concentration of 3-4 μg protein/ml on one side of the BLM formed from azolectin and PS, in the presence of 1 mM $CaCl_2$ on the same side, an increase of conductivity was observed. In Fig. 1 (curve 1) the kinetics of the change in current through the membrane after addition of SV in the presence of Ca^{++} is shown. Conductivity increased 0-6 min after addition of SV by 2-3 orders of magnitude. In most experiments 15-20 min after the beginning of modification, as the conductivity increased, destruction of the membranes took place. In the presence of 1 mM $CaCl_2$ on one side of the membrane conductivity was unchanged for 2 h or more.

Addition of $CaCl_2$ in the same concentrations to the solution already containing SV did not lead to the changes in G described above. This can be explained by competition between two processes induced by Ca^{++}: aggregation of SV and adsorption of SV on the membrane.

On the addition of SV within the concentration range of 4-16 μg protein/ml on one side of a BLM formed from azolectin and PS, an increase in G, dependent on the SV concentration, was observed (Fig. 1, curve 2). The membranes remained stable after modification.

Dependence of the conductivity of a BLM formed from azolectin and PS on the SV concentration is shown in Fig. 2 in the presence of 1 mM $CaCl_2$ and without addition of $CaCl_2$. We consider that the change of conductivity observed in the first case may be due to fusion of SV with BLM. The difference of two orders of magnitude between the changes in G in the presence of Ca^{++} and in its absence, can be explained on the grounds that in the second case the structure of BLM was disturbed during adsorption of SV on the membrane in the absence of their fusion.

The results are evidence that SV can interact with BLM made from azolectin and PS not only in the presence of millimolar concentrations of Ca^{++}, but also in the presence of traces of bivalent cations as impurities.

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