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AGGREGATION AND SWELLING OF RAT BRAIN SYNAPTIC VESICLES

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Secretion of transmitters by nerve endings and of hormones by gland cells takes place by exocytosis, i.e., Ca-dependent interaction between secretory granules and complementary sites on the inner surface of the presynaptic membranes or cell membranes of the gland cells. One result of this heterologous membrane–membrane interaction is the secretion of transmitters or hormones into the extracellular medium [1, 2]. It is claimed [5] that the trigger stage of exocytosis may be facilitation of adhesion of a fixed secretory granule (adherent to the cell membrane during excitation of the cell) to another "transit" secretory granule. Under these circumstances the two granules fuse together into one, from which the transmitters or hormones are later secreted into the extracellular medium. This hypothesis is supported by data of electron microscopy, showing that during depolarization of nerve ending membranes there is some decrease in the number of synaptic vesicles (SV) in the terminals [2]. This hypothesis, besides heterologous membrane–membrane interaction, also postulates a homologous type of interaction, i.e., fusion (aggregation) of secretory granules with one another. The views examined above have stimulated the study of Ca-induced aggregation (fusion) of different secretory granules, in most cases of chromaffin granules of the adrenals and liposomes [2, 3, 5, 6].

In the investigation described below some characteristics of aggregation and swelling of isolated SV from rat brain were studied.

EXPERIMENTAL METHOD

The SV fraction was isolated from whole brain of rats weighing 150-200 g [10]. For this purpose, unpurified synaptosomes were obtained from a 10% brain homogenate (0.32 M sucrose, 20 mM Tris-HCl, pH 7.4, 0.1 mM EDTA) after removal of the nuclei (10,000g, 10 min), subjected to osmotic shock, after which the residue was suspended in distilled water (4 ml of water to residue obtained from 1 g of brain tissue). After freezing at −20°C and thawing the suspension was centrifuged at 18,000g for 30 min and the supernatant was then centrifuged at 120,000g for 40 min. The residue thus obtained was the SV fraction. The fraction was suspended in 0.25 M sucrose, containing 20 mM Tris-HCl, pH 7.4, and kept for 1 week at −10°C. The preparation, frozen once only, was used in the work. Protein was determined by Lowry's method.

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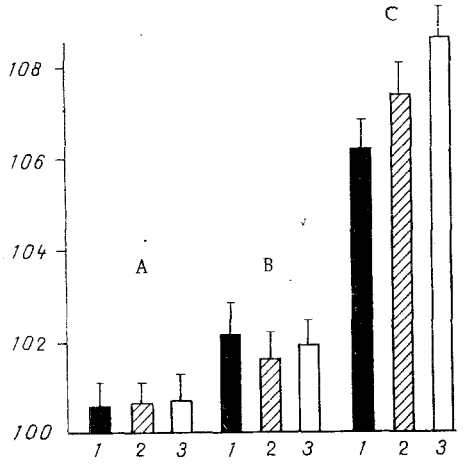

Fig. 1

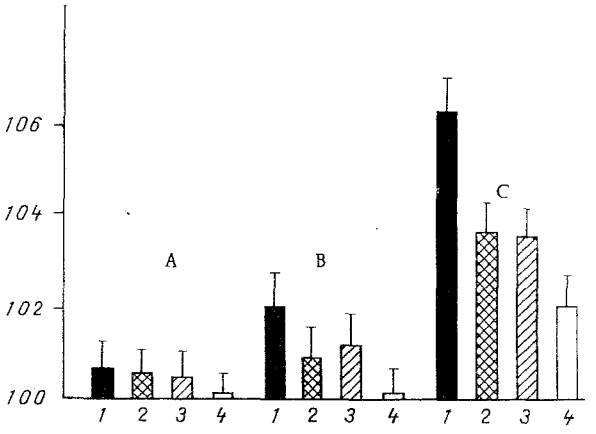

Fig. 2

Fig. 1. Effect of Ca^{++} on aggregation of rat brain SV. Ordinate, I_{SC} (change in I_{SC} after addition of Ca^{++}, in % of initial value). A, B, C) 10^{-5}, 10^{-4}, and 10^{-3} M $CaCl_2$ respectively, 1, 2, 3) 0.05, 0.1, and 0.2 mg protein/ml respectively. Here and in Figs. 2-4 mean results of 5-7 experiments given.

Fig. 2. Effect of Mg-ATP on Ca-(Mg)-induced aggregation of suspension of rat brain SV. Ordinate, change in I_{SC} after addition of bivalent cations (in % of initial value). A, B, C) Concentration of bivalent cations 10^{-5}, 10^{-4}, and 10^{-3} M respectively. a) Ca^{++}; 2) Mg-ATP + Ca^{++}; 3) Mg^{++}; 4) Mg-ATP + Mg^{++}. Effect of aggregation during combined action of Mg-ATP (1 mM) and cations was calculated relative to I_{SC} measured after addition of Mg-ATP.

Aggregation of SV in keeping medium (2 ml) was recorded by the change in intensity of scattering of light (I_{SC}) at an angle of 90° at 37°C, with mixing, on an MPS-4 spectrofluorometer (Hitachi, Japan). The initial value of I_{SC} of the SV suspension was unchanged during incubation for 60 min in 0.25 M sucrose, 20 mM Tris-HCl, pH 7.4, 100 μg/ml. The change in aggregation (or volume) of SV under the influence of various factors was estimated as a percentage of the original value of I_{SC}. In all experiments the dilution effect was taken into account on addition of the substances. ATP-Na_2 (neutralized to pH 7.4) was obtained from Reanal, Hungary, colchicine and cytochalasin B were obtained from Serva, West Germany, and dissolved in 96% ethanol (final ethanol concentration in the samples 1%); dicyclohexylcarbodiimide (DCCD) and carbonylcyanide-m-chlorophenylhydrazone (CCCP) were obtained from Serva (West Germany), and also dissolved in 96% ethanol (final ethanol concentration 1%). Concentrations of Ca^{++} and Mg^{++} solutions were determined by complexometric titration [4].

EXPERIMENTAL RESULTS

With an increase in the protein concentration in the SV suspension from 0.05 to 0.5 mg/ml a linear increase in the value of I_{SC} was observed (in medium without Ca^{++}), and this could be evidence of the absence of "self-aggregation" of the vesicles.

Ca^{++} ions induced aggregation of brain SV within the concentration range of 10^{-5}–10^{-3} M (Fig. 1); the effect was most marked, moreover, for high Ca^{++} concentrations (1 mM). If Ca^{++} was used within the concentration range of 10^{-4}–10^{-5} M the intensity of SV aggregation was independent of the protein concentration of the vesicles (50-200 μg/ml). However, with higher Ca^{++} concentrations (1 mM) the degree of Ca-induced aggregation of SV depended on the protein concentration of the vesicles. The increase in I_{SC} under the influence of bivalent cations, incidentally, reached a maximum (the curve flattened out on a plateau) in the course of 5-7 min. All values shown in Figs. 1-4 thus relate to 7 min after additions of the cations.

In the next experiments the protein concentration of the SV suspension was 100 μg/ml. It will be clear from Fig. 2 that the effect of Ca^{++} and Mg^{++} in a concentration of 10^{-4}–10^{-5} M on aggregation of SV was almost identical. Meanwhile, if the concentration of bivalent cations was 10^{-3} M the degree of SV aggregation was significantly higher in the presence of Ca^{++} than in the presence of Mg^{++}. It was found that Ca-induced aggregation of brain SV with Ca^{++} in a concentration of 1 mM took place far more intensively at pH 5.0 than at pH 7.4. This was perhaps due to neutralization of the negative charge on the SV membrane surface at neutral pH

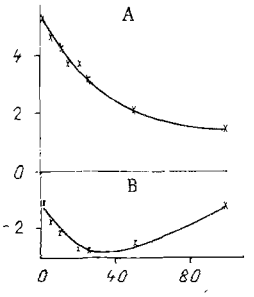

Fig. 3

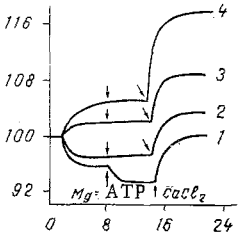

Fig. 4

Fig. 3. Dependence of aggregation (A) and degree of swelling (B) of SV suspension on KCl concentration. Abscissa, KCl concentration (in mM); ordinate, change in I_{SC} (in % of initial). Ionic strength of solution remained constant on account of a corresponding decrease in sucrose concentration. Results of two experiments shown. Swelling was induced by addition of Mg-ATP (1 mM), aggregation by Ca^{++} (1 mM) against the background of Mg-ATP.

Fig. 4. Action of H^+-ATPase blockers DCCD and CCCP on swelling and aggregation of suspension or rat brain SV. Abscissa, incubation (in min); ordinate, I_{SC} (in %). 1) Ethanol (1%); 2) Mg-ATP (1 mM); 3) CCP (40 μM); 4) DCCD (50 μM).

values. As has already been stated, Ca-induced aggregation of secretory granules is a known fact. In particular, it has been described for rat brain SV [3], the electric organ of the skate [6], and the neurohypophysis [5]. It is considered that Ca^{++} ions neutralize the charge on the SV membrane, bind with negative charges of membrane proteins, phospholipids, and mucopolysaccharides, and mainly with the COOH groups of phospholipids – the principal components of the SV membrane, and they thus screen electrostatic interaction between SV [2, 3]. Exocytosis of transmitters (or hormones) is an exclusively Ca-dependent process, during which Ca^{++} ions exert their action in concentrations of about 1 μM, against the background of a high Mg^{++} level (about 1-3 mM). Although in the present experiments some selectivity of action of Ca^{++} was observed compared with Mg^{++} in relation to aggregation of rat brain SV, this can hardly be regarded as physiological, for it was observed when the cations were present in quite high concentrations.

Considering that exocytosis is an Mg-ATP-dependent and Ca-induced process [3], in the next series of experiments the Ca-induced aggregation of brain SV was studied in the presence of Mg-ATP. A preliminary study was made of the action of Mg-ATP on the osmotic properties of SV. In the present experiments addition of Mg-ATP (0.5-2.0 mM) caused a decrease in I_{SC} ($P < 0.05$) of the SV suspension, indicating swelling of the SV. This process of an increase in volume of the brain vesicles was exhibited most intensively in the presence of 1-2 mM Mg-ATP. Under the influence of Mg-ATP, I_{SC} reached minimal values after 1-2 min, and remained at that level during 20 min of incubation. Facts of this kind were observed previously for rat brain SV [3] and for chromaffin granules in the adrenals [7, 9]; this phenomenon, moreover, is exhibited mainly in medium containing penetrating anions (Cl^-). It has been suggested [7, 9] that Mg-ATP-induced swelling in chromaffin granules is coupled with functioning of the H^+-pump, transport of anions into the granules, and subsequent water inflow. This hypothesis also is confirmed by the present experiments.

It was shown that Mg-ATP-induced swelling is increased in the presence of KCl to 25-50 mM, and that a further increase in the KCl concentration to 100 mM abolished this effect (Fig. 3). Under these same conditions, however, addition of Ca^{++} (1 mM) against the background of Mg-ATP (1 mM) sharply reduced the degree of SV aggregation with an increase in the KCl concentration from 5 to 100 mM. We know [6, 8] that high KCl concentrations ($\geq$ 50 mM) can induce aggregation (dimerization) of the chromaffin granules of the adrenals and SV of the electric organ of the skate. It can accordingly be postulated that the decrease in Ca-induced SV aggregation (against the background of Mg-ATP) in the region of low KCl concentrations (up to 25-50 mM) can be

explained on the grounds that "swollen" vesicles do not aggregate so well. KCl-induced SV aggregation ($\geq$ 50 mM) makes the vesicles insusceptible to subsequent additional aggregation on the addition of Ca^{++}. In this connection it must be noted that if the originally isolated SV were suspended in 0.125 M KCl, 20 mM Tris-HCl, pH 7.4, these vesicles gave virtually none of the effects described above on the addition of 1 mM Ca^{++} (without Mg-ATP) or of 1 mM Mg-ATP (without Ca^{++}). In order to demonstrate the role of H^+-ATPase in swelling of brain SV, the action of known blockers of H^+-ATPase (DCCD and CCCP) was studied on this process when induced by addition of Mg-ATP. Accordingly, a preliminary study was undertaken of the action of these substances themselves on I_{SC} of the brain SV suspension. It was found that addition of 1% ethanol to the samples caused a reduction in the value of I_{SC}, possible evidence in principle of partial swelling of SV. Addition of CCCP up to a final concentration of 20-40 μM (allowing for the effect of ethanol) caused some increase in I_{SC}, whereas the addition of DCCD, another blocker of the H^+-pump, in a concentration of 50-100 μM (allowing for the effect of ethanol) led to a greater increase in I_{SC} of the SV suspension (Fig. 4).

The experiments showed that the blockers of the H^+-pump inhibited Mg-ATP-induced swelling virtually completely, and this confirmed the hypothesis put forward above.

Let us now turn to experiments to study Ca-induced aggregation of the SV suspension. It was found that Mg-ATP (1 mM) leads to a decrease in SV aggregation induced by addition of both Ca^{++} and Mg^{++} (Fig. 2). A particularly marked effect was observed with a high concentration of the cations (1 mM). Incidentally, after the SV had aggregated in the presence of high Ca^{++} concentrations (1 mM) without preliminary addition of Mg-ATP, they preserved their ability to swell in response to subsequent addition of Mg-ATP (1 mM).

The cytostatics colchicine and cytochalasin B (0.5-1.0 mM and 10^{-5} M respectively), used in the work, if preincubated for 30 min, affected neither Mg-ATP-induced swelling of SV suspensions nor Ca-induced aggregation (1 mM Ca^{++}), when studied after addition of Mg-ATP. These experiments evidently indicate that contractile cytoskeletal structures associated with brain SV membrane do not participate directly in the processes studied.

It must be pointed out that Ca-induced aggregation of the brain SV suspension (in the presence of 1 mM Ca^{++}) is increased by 2-2.5 times after preliminary addition of DCCD and Mg-ATP (Fig. 4).

When the physicochemical properties of brain SV are studied or exocytosis is simulated in a model using isolated SV and brain synapses, it is thus necessary to take into account such important parameters as swelling and aggregation of the vesicles and the conditions under which they are manifested.

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