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INTERACTION BETWEEN ACTIN-LIKE BRAIN PROTEIN AND ISOLATED SYNAPTIC VESICLES

Yu. G. Sandalov, R. N. Glebov, G. N. Kryzhanovskii,* V. I. Shvets, and G. V. Tolstikova

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Mg-ATP was shown to increase the light diffusion of a suspension of rat brain synaptic vesicles (SV) in the presence of rat brain actin-like protein (ALP) (superprecipitation reaction). ALP increases the Mg-ATPase activity of SV and also the liberation of endogenous noradrenalin from SV of bovine hypothalamus, which is abolished by cytochalasin B. Glycolipids (gangliosides and cerebrosides) inhibit the superprecipitation reaction. The results are examined from the standpoint of the contractile hypothesis of mediator secretion.

KEY WORDS: brain actin-like protein; synaptic vesicles; secretion of mediators.

Interest in the study of contractile proteins of nonmuscular origin, especially proteins of brain tissue, has recently strengthened. For instance, an actin-like protein (ALP) has been localized in brain nerve endings mainly in presynaptic membranes (pre-SM); a myosin-like protein (MLP) mainly in synaptic vesicles (SV); and troponin- and tropomyosin-like protein in free SM and to some extent also in SV [5, 6]. In an enriched rat brain synaptic membrane fraction 11% of the total protein has been found to consist of ALP [11].

According to the contractile hypothesis of mediator secretion [2, 5], the act of secretion of mediators by nerve endings is the result of interaction between the MLP of the SV membranes with ALP which is a structural component of pre-SM. The formation of an actomyosin-like protein complex (AMLP) is initiated by an increase in the Ca^{++} concentration in nerve endings during their depolarization. The act of secretion also involves transport of SV to the active zone of pre-SM, possibly on account of interaction of the vesicles with microtubules and neurofilaments. Data on the blocking of secretion of various mediators by mitotic alkaloids [1, 2] can be regarded as confirmation of the contractile hypothesis. The effect of potentiation of liberation of exogenous mediator into the incubation medium from isolated SV, previously loaded with [^{14}C]-glutamate, on interaction between SV and brain ALP preparation or actin from skeletal muscles, containing Ca-sensitive components of the contractile system – in these experiments the formation of an AMLP complex was tested by finding increased Mg-ATPase activity of the SV fraction [13].

In the present investigation an attempt was made to test the contractile hypothesis on a new model: liberation of endogenous noradrenalin (NA) mediator from SV during the formation of an AMLP complex, monitored by measuring Mg-ATPase activity and changes in light diffusion of the SV suspension in the presence of ALP. The role of glycolipids as possible regulators of formation of the AMLP complex also was studied.

EXPERIMENTAL METHOD

A preparation of ALP containing Ca-sensitive proteins also was isolated from bovine cerebral cortex [12]. Tests showed that the ALP preparation did not possess ATPase activity and did not contain lipid components. The SV fraction was isolated from bovine hypothalamus or from whole rat brain (without the cerebel-

*Corresponding Member of the Academy of Medical Sciences of the USSR.

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TABLE 1. Effect of Bovine Brain ALP on Mg-ATPase Activity of SV Fraction of Rat Brain (M ± m)

Experimental conditions	Mg-ATPase activity, %		
	- ALP	- ALP (50 μg protein)	+ ALP (500 μg protein)
SV (100 μg protein) + Ca^{++}	100±5 (12)	91,9±12,2 (12)	134±7* (6)
SV (100 μg protein) − Ca^{++}	113±8 (9)	119,3±5,8 (8)	122±10 (6)

Legend. Mg-ATPase activity of SV fraction was 7.9 μmoles P_i/mg protein/h at 37°C. Incubation medium (in mM): KCl 50, Tris-HCl (pH 7.4) 20, ATP-Na_2 3, $MgCl_2$ 5, $CaCl_2$ 0.1; $-Ca^{++}$ signifies addition of 0.1 mM EGTA. Asterisk indicates value differing significantly from control at $P < 0.05$ level. Here and in Table 2 the number of experiments is shown in parentheses.†

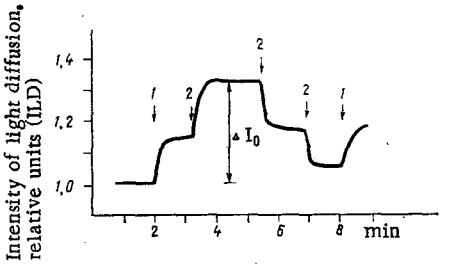

Fig. 1. Changes in intensity of light diffusion of rat brain SV suspension in presence of bovine brain ALP (superprecipitation reaction). Incubation medium (in mM): KCl 50, Tris-HCl (pH 7.4) 20, ALP 80-90 μg protein per sample, SV 100 μg protein per sample. Volume of sample 2.5 ml, 22°C. Arrows indicate additions of: 1) $MgCl_2$ 1.0 mM, 2) ATP-Na_2 1.25 mM.

lum) by the method in [8]. For this purpose the fraction of unpurified synaptosomes was treated by hypo-osmotic shock (with 0.5 ml deionized water to 1 mg protein of the fraction) followed by a single freezing (−20°C) and thawing. The residue after centrifugation (18,000g, 30 min) was discarded. The SV fraction was obtained by centrifugation (120,000g, 40 min) of the supernatant and was suspended in 100 mM KCl and 20 mM Tris-HCl, pH 7.4; the SV fraction was used on the day of its isolation.

ATPase activity was determined by measuring accumulation of inorganic phosphorus in the incubation (20 min, 37°C) medium by the method of Lowry and Lopez. Protein was determined by Lowry's method. The intensity of light diffusion was measured at an angle of 90° at 22°C in a Hitachi-204 (Japan) spectrofluorometer at 520 nm. The total fraction of gangliosides [14] and cerebrosides [10] was isolated from bovine brain. Glycolipids were emulsified in water. NA was determined by a fluorometric method [9].

EXPERIMENTAL RESULTS

It will be clear from Table 1 that interaction between SV and ALP in medium containing Ca^{++} (0.1 mM) led to a considerable and significant increase in Mg-ATPase activity of the vesicles fraction, possibly evidence of the formation of an AMLP complex. This conclusion was drawn from the test for formation of muscle actomyosin and actin in the presence of Ca-sensitive protein coenzymes. The addition of ALP to SV

†Table 2 does not appear in the Russian original – Consultants Bureau.

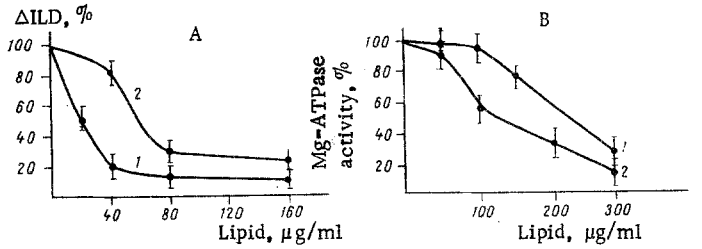

Fig. 2. Action of gangliosides (1) and cerebrosides (2) on Mg-ATPase of SV fractions (A) and on superprecipitation reaction (B). Ordinate: A) change in Mg-ATPase activity (in %); B) change in intensity of light diffusion (in %; ΔILD) on addition of 1 mM $MgCl_2$ and 1.25 mM ATP-Na_2 to mixture of SV and ALP; change in light diffusion in absence of lipids (ΔI_0 in Fig. 1) taken as 100%; abscissa, concentration of glycolipids, in μg/ml. For experimental conditions see Table 1 and Fig. 1.

in the presence of EGTA caused virtually no increase in Mg-ATPase activity. It can tentatively be suggested that formation of the AMLP complex in these experiments was Ca-dependent in character, in agreement with data in the literature [13].

Formation of the AMLP complex also was confirmed by the superprecipitation reaction, tested by determining the change in intensity of light diffusion at an angle of 90°. As Fig. 1 shows, successive addition of microvolumes of $MgCl_2$ and ATP-Na_2 (10 μl of each, pH 7.4, final concentration 1.0 and 1.25 mM, respectively) caused a marked increase in the intensity of light diffusion. An increase in the ATP concentration in the medium led to a decrease in the intensity of light diffusion, possibly indicating dissociation of the AMLP complex which had been formed. This reversible reaction can be shifted toward formation of the complex by the addition of high concentrations of Mg^{++}. In this case the AMLP complex was evidently formed on the surface of the SV membranes at sites of attachment (incorporation) of MLP. Incidentally, the superprecipitation reaction between SV and the ALP preparation is described here for the first time. Previous electron-microscopic investigations demonstrated the formation of filaments of F-actin from skeletal muscles on the surface of chromaffin granules isolated from bovine adrenal glands [7]. The same authors observed brightening of the chromaffin granules, from which they concluded that catecholamines passed out of the granules into the incubation medium.

In the next series of experiments the possibility of liberation of endogenous mediator (NA) from SV isolated from bovine hypothalamus was studied. It was shown first that spontaneous liberation of NA from hypothalamic SV into the medium during incubation with bivalent ions and ATP (without ALP) amounts to 45%. Addition of ALP led to an increase (by 30%) in the amount of NA liberated into the incubation medium. The incubation medium has the following composition (in mM): KCl 250, Tris-HCl (pH 7.4) 20, ATP-Na_2 0.2, $MgCl_2$ 0.2, $CaCl_2$ 0.1; SV 90-120 μg protein per sample, ALP 70-90 μg protein per sample. The volume of the sample was 4 ml; the temperature was 37°C. The formation of an AMLP complex during contact between SV and the ALP preparation, evidently by causing structural changes in the vesicle membranes, led to liberation of portions of mediator into the incubation medium (NA output 130 ± 10%; n = 9). Experiments with the mitotic alkaloid cytochalasin B (10 mg/ml) showed that this mitotic poison, by binding selectively with actin, inhibited NA liberation (output 112 ± 12%; n = 6) on account of inhibition of formation of the AMLP complex. Mitotic alkaloids (colchicine, vinblastin, and cytochalasin B) are known [4] to block the secretion of biogenic amines during potassium depolarization of isolated nerve endings (synaptosomes).

Interaction between isolated SV and contractile brain proteins can thus be used as a model of mediator secretion in vitro and the regulation of this process.

The writers postulated previously [1, 2] that in a resting state the ALP in pre-SM is in the form of a complex with glycolipids but during excitation the ALP is liberated from its "lipid guardianship" and becomes accessible for contact with SV. This hypothesis is based on the selective binding of isolated AMLP with glycolipids observed in [3]. It is also known [15] that during electrophoresis of synaptic membrane proteins in polyacrylamide gel a protein fraction determined as ALP was identified from histochemical data as a glycoprotein.

To test this hypothesis, the effect of brain gangliosides and cerebrosides on interaction between SV and ALP was studied. As Fig. 2A shows, gangliosides and cerebrosides in high concentrations (100-300 μg/ml) considerably inhibited the Mg-ATPase of the SV fraction (without addition of ALP). Meanwhile, incubation (5 min, 20°C) of ALP with glycolipids in a concentration of 20-50 μg/ml, i.e., in concentrations in which these lipids did not affect the Mg-ATPase of the SV fraction (see Fig. 2A), inhibited the superprecipitation reaction (Fig. 2B). In the latter case the inhibitory effect was more marked for gangliosides than for cerebrosides. When bound with glycolipids, ALP thus loses its ability to interact with SV, i.e., to form an AMLP complex. The results of these investigations indicate that glycolipids may regulate contact between pre-SM and SV membranes and the process of exocytosis.

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