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ANTI-ACTOMYOSIN

INFLUENCE ON ADHESIVE BEHAVIOUR OF EUKARYOTIC CELLS AND OF CUVIERIAN TUBULES

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SUMMARY

The effect of antisera against chicken gizzard smooth-muscle actomyosin and against pectoralis striated-muscle actomyosin on adhesive behaviour of eukaryotic cells (from sea urchin embryos and from a silicious sponge) and of Cuvierian tubules has been studied. The results with sea urchin cells, which require divalent cations for aggregation, showed that antiserum to chicken gizzard smooth-muscle actomyosin inhibited reaggregation of trypsin-treated cells better than mechanically dissociated cells, while anti-chicken pectoralis striated-muscle had no effect. Primary reaggregation of trypsin-dissociated sponge cells, in the presence of calcium and magnesium, is also inhibitable by anti-gizzard smooth-muscle but not by anti-pectoralis striated-muscle. Anti-gizzard smooth-muscle had no effect on secondary reaggregation of sponge cells mediated by a soluble aggregation factor. Anti-gizzard smooth-muscle inhibited Cuvierian tubule adhesion.

Actomyosin antibodies have been shown to inhibit aggregation of embryonic chick muscle and liver cells [1, 2]. From this observation the hypothesis has been propounded, that a surface-localized myosin-type protein serves a function in the regulation of cell adhesion [3, 4]. There is some evidence that myosin-like proteins are located at the surface of trypsinized cells from eukaryotic organisms [5–8]. Due to the findings that rabbit antisera against human actomyosins have been found to be type specific [9] but not species specific [1], rabbit anti-actomyosins seem to be an appropriate tool to block the function of cell-surface-located myosin-like proteins on cells of different organisms [2, 7].

It is the aim of this investigation first, to determine the action of anti-actomyosin on membrane adhesion and second, to check whether the adhesion inhibiting influence of anti-actomyosin is dependent on a pretreatment of cell membrane with

the proteolytic enzyme trypsin. As a test model in which hydrophobic interaction of biological membranes is determined to play a role we used the Cuvierian tubules of the sea cucumber *Holothuria forskali* [10, 11]. Then for cell-cell interactions caused in the presence of Ca^{2+}, single cells from sea urchin embryos (*Sphaerechinus granularis*) [12, 13] and from a silicious sponge (*Geodia cydonium*) [14, 15] were applied. It can not be conclusively said whether the underlying mechanisms are only restricted to the respective model or whether they may be active in each of the models to different strength. Undoubtedly these two bonding interactions are not the sole cause of cell adhesiveness, as reviewed e.g. by Curtis, ref. 16.

Antisera against actomyosin from chicken pectoralis striated-muscle (termed hereafter: anti-pectoralis striated-muscle) and chicken gizzard smooth-muscle (anti-gizzard smooth-muscle) were prepared according to Kemp et al. [2]. Control rabbit γ-globulin was purchased from Serva, Heidelberg (G.F.R.). Before use lyophylized anti-pectoralis striated-muscle, anti-gizzard smooth-muscle and control γ-globulin were dissolved in calcium- and magnesium-free sea water [14]; insoluble material was removed by centrifugation (11 000 $\times g$; 10 min; 15 °C).

Influence on membrane adhesion in the presence of Ca^{2+}

As reported elsewhere [17, 14] dissociated cells from sea urchin embryos as well as the ones from sponges form aggregates only in the presence of divalent cations, especially of Ca^{2+}. As shown in Table I in the presence of Ca^{2+} the number of single cells from blastulae of the sea urchin *S. granularis* decreases during reaggregation in the case of mechanically dissociated cells from $1 \cdot 10^5$/ml at the beginning of reaggregation to $2.6 \cdot 10^4$/ml at the end of the observation time; in the case of chemically dissociated cells the number of single cells drops from $1 \cdot 10^5$/ml to $3.1 \cdot 10^4$/ml. The presence of control γ-globulin during the reaggregation process does not alter the rate of aggregation. Furthermore anti-pectoralis striated-muscle also fails to inhibit cell aggregation. In contrast to these findings anti-gizzard smooth-muscle inhibits aggregation of sea urchin cells markedly. The inhibition is considerably more pronounced in the case of aggregation of chemically (by the use of trypsin) dissociated cells than in the assay with mechanically dissociated cells; using 250 μg anti-gizzard smooth-muscle 53 % of the chemically dissociated cells remain as single cells compared with 35 % in the case of mechanically dissociated cells.

As reported previously [14, 15] reaggregation of dissociated sponge cells can be subdivided into a first event, the formation of primary aggregates in the absence of a soluble aggregation factor and a second process, the formation of secondary aggregates from the primary aggregates, initiated by the aggregation factor. During the secondary aggregation phase the number of aggregates drops considerably. The ability of chemically dissociated (trypsinized) sponge cells to form primary aggregates is not influenced by control γ-globulin and anti-pectoralis striated-muscle, however it is strongly inhibited by anti-gizzard smooth-muscle (Table II). In the presence of 50 (250) μg anti-gizzard smooth-muscle/ml the number of primary aggregates is reduced to 76 (65) %. The formation of secondary aggregates from primary aggregates in the presence of a soluble aggregation factor is not affected significantly by anti-gizzard smooth-muscle (Table II).

TABLE I

Influence of control γ-globulin, and the anti-actomyosins anti-gizzard smooth-muscle and anti-pectoralis striated-muscle on the number of single cells in a suspension of dissociated cells from blastulae (14 h old) of *S. granularis*. Dissociation was performed either chemically with 30 μg/ml trypsin (2 units/mg; Merck, Darmstadt, Germany) in calcium- and magnesium-free artificial sea water (30 min, 20 °C) or mechanically by dispersing in a Dounce homogenizer in calcium- and magnesium-free artificial sea water containing 20 mM ethylene-glycol bis(β-aminoethyl ether)-*N*,*N*-tetraacetic acid (EGTA) (Sigma, St. Louis, U.S.A.) as described [12]. For reaggregation the disaggregated cells were transferred into filtered sea water and aggregation was performed in a 3 ml aliquot of the cell suspension ($1 \cdot 10^5$ cells/ml) in 20 ml glass tubes using the roller technique [14]. Aggregation was performed at 37 °C for a period of 90 min. As a measure for aggregation the number of single cells was estimated at the end of the aggregation period by microscopical counting. The measurements are linear with cell number [12, 13]. Number of determinations: 10; significance values (*P*) between control and test are indicated.

	Concentration (μg/ml)	Number of single cells ($\times 10^4$/ml) at the end of the incubation			
		Mechanically dissociated cells	*P*	Chemically dissociated cells	*P*
None		2.6		3.1	
Control γ-globulin	50	2.7	> 0.2	3.0	> 0.2
	250	2.6	> 0.2	3.1	> 0.2
Anti-actomyosin from gizzard smooth-muscle	50	3.3	< 0.005	4.3	< 0.001
	250	3.5	< 0.001	5.3	< 0.001
Anti-actomyosin from pectoralis striated-muscle	50	2.8	> 0.2	3.3	> 0.2
	250	2.8	> 0.2	3.4	> 0.2

TABLE II

Effect of control γ-globulin, anti-gizzard smooth-muscle and anti-pectoralis striated-muscle on primary and secondary aggregation of chemically dissociated *Geodia* cells. Dissociation of sponge tissue into separate cells was performed with 100 μg/ml trypsin in calcium- and magnesium-free artificial sea water as described [14]. For primary aggregation washed cells were placed into calcium and magnesium containing artificial sea water [14]; for secondary aggregation the highly purified aggregation factor, isolated from *G. cydonium* [15], was added in a concentration of $1.0 \cdot 10^3$ A.U./ml. The assays were performed in 20 ml glass tubes and contained 3 ml of a suspension with $25 \pm 5 \cdot 10^6$ single cells/ml; aggregation was performed at 20 °C for 120 min using the roller technique [14]. As a measure for primary aggregation, the number of primary aggregates has been determined [14]; in the case of secondary aggregation, the total number of aggregates (primary + secondary aggregates) has been evaluated [14]. The measurements of formation of aggregates are linear with time [14]. Number of determinations: 10; significance (P) is indicated.

	Concentration (μg/ml)	Primary aggregation		Secondary aggregation	
		($\times 10^5$ primary aggregates/assay)	P	(aggregates/assay)	P
None	—	6.6	—	228	—
Control γ-globulin	50	6.4	>0.2	243	>0.2
	250	6.5	>0.2	237	>0.2
Anti-actomyosin from gizzard smooth-muscle	50	5.0	<0.005	181	0.015
	250	4.3	<0.001	174	0.01
Anti-actomyosin from pectoralis striated-muscle	50	5.9	>0.2	195	>0.2
	250	6.2	>0.2	206	>0.2

Influence on membrane adhesion of Cuvierian tubules

In a previous paper [11] we could demonstrate that the outer surfaces of the two concentric cylinders of the Cuvierian tubules from *H. forskali* stick strongly especially to hydrophobic surfaces submersed or outside the water. The investigation of the adhesion behaviour of the Cuvierian tubules revealed that adhesiveness is also caused by hydrophobic interactions; ion bonds seem to be involved in adhesion only to a very limited extent, if at all [11, 18]. The adhesiveness of the Cuvierian tubules is not affected significantly by control γ-globulin and anti-pectoralis striated-muscle; in contrast to this result anti-gizzard smooth-muscle reduces adhesiveness up to 43 % (Table III).

The presented results show that anti-pectoralis striated-muscle does not affect the aggregation behaviour of dissociated cells from sea urchin embryos and from a sponge while anti-gizzard smooth-muscle causes a significant reduction of aggregation. The results are in accordance with previous results [1, 2]. The finding that after a pretreatment of the cells with the protease trypsin, the anti-actomyosin effect is enhanced to 171 % is remarkable. Future experiments must show whether during the trypsin treatment the amount of uncovered myosin-type protein molecules on the cell surface increases. With respect to the idea that myosin at the cell surface is important in cell adhesion [3, 4, 7], the result presented may indicate that myosin does not play an exclusive role in the aggregation factor mediated adhesion. This assumption is supported by previous findings [15] also showing that Sepharose beads, coupled with the sponge aggregation factor by the application of the carbodiimide method, show

TABLE III

Dependence of adhesiveness of Cuvierian tubules on control γ-globulin, anti-gizzard smooth-muscle and anti-pectoralis striated-muscle. A single Cuvierian tubule was incubated in sea water (20 ml) for 30 min at 16 °C with the serum protein fractions; subsequently adhesiveness was measured as described before [11]. The tubules are hooked to a spring balance; thus they are hanging downwards with both their free ends. Then the free-hanging doubled threads were brought into contact with a glass slide (75 × 25 mm) coated with a paraffin layer. The glass slide with the tubules adhering to the paraffin coat is moved downwards (4 cm/s), causing a deflection of the spring balance. The force (expressed in g/wt) related to this tension is a measure for adhesiveness of the doubled Cuvierian tubules. The measurements are linear with time [11]. Each value (mean) comes from 10 parallel assays; significance values (P) are given.

	Concentration (μg/ml)	Adhesiveness		
		(g/wt)	(in per cent)	P
None	—	31.1	100	—
Control γ-globulin	50	29.8	96	>0.2
	100	31.9	103	>0.2
	250	33.5	108	>0.2
Anti-actomyosin from gizzard smooth-muscle	50	22.0	71	<0.005
	100	17.4	56	<0.001
	250	13.3	43	<0.001
Anti-actomyosin from pectoralis striated-muscle	50	32.2	104	>0.2
	100	30.1	97	>0.2
	250	28.8	93	>0.2

"aggregation"; Sepharose beads consist of dextran and do not contain actomyosin [19]. Not only membrane adhesiveness caused primarily by ion bondings (cells from sea urchin embryos and from sponges) but also membrane adhesiveness provoked primarily by hydrophobic interactions, as has been shown with Cuvierian tubules, is strongly reduced by the anti-actomyosin anti-gizzard smooth-muscle, which might block myosine sites involved in adhesive processes. In contrast to the eukaryotic cells where myosin-type protein has been detected at the cell surface [5, 6], no data are available about the presence of actomyosin in the Cuvierian tubules. However it is most likely that the Cuvierian tubules do contain these proteins due to the findings that the tubules show a high mobility [10] and contain myofibrils (electron microscopical observation; unpublished).

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