Journal of Supramolecular Structure 3:391–400 (1975)

SURFACE MODIFICATIONS EVOKED BY ANTIDIURETIC HORMONE IN ISOLATED EPITHELIAL CELLS: Evidence from Lectin Probes

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Epithelial cells (80–90% "granular" type) were isolated from urinary bladders of *Bufo marinus* and *Rana catesbiana*. The inhibitory effect of α-methyl-D-mannoside on fluorescein-labeled concanavalin A (Con A) binding to these cells indicates that they possess specific binding sites for Con A. The lectin also mediates adsorption of erythrocytes to these cells. Both Con A binding and Con A-mediated hemadsorption to epithelial cells are depressed at 4°C, as compared with cells maintained at 22°C. Elevation of temperature to 37°C, however, enhances hemadsorption independently of alterations in lectin binding. Treatment of cells with antidiuretic hormone (ADH) at 22°C followed by 15 min of incubation at 22° or 37°C before exposure of cells to Con A promotes increments in Con A-mediated hemadsorption, but not in lectin binding, at 22° or 37°C. These hormonal effects are not significant when hemadsorption is assayed at 4°C. Treatment of cells with another octapeptide, angiotensin, elicits a small, but significant, increment in hemadsorption to epithelial cells which is likewise uninfluenced by quantitative changes in lectin binding. Collectively, these data and other independent observations suggest that treatment with octapeptide hormones acts to enhance the redistribution and aggregation of lectin-binding proteins in the membranes of granular epithelial cells from amphibian urinary bladder. Such changes, in turn, may contribute to the alterations in membrane transport properties which characterize the hormonal response.

INTRODUCTION

It is well known that antidiuretic hormone (ADH) increases the permeability of amphibian urinary bladders to water and small polar solutes (1, 2). In addition, ADH elicits a general, nonselective increase in the permeation of lipophilic solutes and a decrease in the discrimination between straight- and branched-chain isomers (3). Collectively, these results lead to the assumption that the hormone increases the permeability of amphibian urinary bladder epithelium by increasing the fluidity of the cell membranes.

Alterations in the physical state of membrane lipids are known to influence nonelectrolyte permeation (see Wilson et al. [4]) and redistribution of membrane surface components (5, 6). Aub et al. (7) were the first to demonstrate clearly that plant lectins tend to agglutinate transformed cells more efficiently than their normal parent cells. Since differences in the number of lectin-binding sites are inadequate to explain the differences in agglutinability (6, 8), the clustering of lectin-binding sites and the resultant increase in

Manuscript received February 25, 1975, revision accepted June 4

agglutinability of transformed cells is considered to be a consequence of increased lateral movement of lectin-binding components in the plane of the membrane (cf. Nicolson [9]). Using fluorescein-labeled concanavalin A (Con A), we have investigated the binding of this lectin as well as Con A-mediated hemagglutination to epithelial cells in the presence and absence of hormones known to influence the function of amphibian bladder epithelium.

MATERIALS AND METHODS

Isolated epithelial cells (80–90% "granular" type) were obtained from the urinary bladders of *Bufo marinus* and *Rana catesbiana* (Mogul-Ed Corp., Oshkosh, Wis.) with procedures employing collagenase, as previously described by Pietras (10) and Pietras and Szego (11).

Preparation of Red Blood Cell Suspensions

Whole blood was obtained from the appropriate species by cardiac puncture following intraventricular injection of the sodium salt of heparin (0.1 ml, 100 IU) on the day of experiment. The blood was centrifuged at 400 × g for 10 min. The packed erythrocytes were then diluted to 1% vol/vol with divalent cation-free Ringer's solution (DCFRS) otherwise constituted as follows: 104.5 mM NaCl, 2 mM KCl, 1 mM sodium pyruvate, and buffered at pH 7.4 with 2.125 mM Na_2HPO_4/0.375 mM NaH_2PO_4. Aliquots of the resultant erythrocyte suspension were then used in the hemadsorption experiments as described below.

Measurement of Concanavalin A-Mediated Hemadsorption to Isolated Epithelial Cells from Amphibian Bladder

Epithelial cells isolated from amphibian urinary bladders were suspended in normal Ringer's solution (with the divalent cations, 1 mM $CaCl_2$ and 1 mM $MgSO_4$). 5-ml aliquots ($\sim 2.5 \times 10^6$ cells) were incubated for 15 min in the presence or absence of added reagents at 22°C in a stop-flow chamber described elsewhere (12). The bottom of the chamber was fitted with a Nuclepore GE-800 filter with an 8.0-μm pore size that allowed passage of amphibian erythrocytes while permitting retention of the epithelial cells. The cells were washed twice in the chamber with complete Ringer's solution and incubated for 3 min with selected concentrations of unmodified Con A in Ringer's solution. The suspensions were then washed five times with DCFRS to reduce divalent cation-mediated cell aggregation and incubated for 7 min with amphibian erthrocytes in DCFRS. Finally, the cells were washed again with DCFRS five times, solubilized in 5% sodium dodecyl sulfate (wt/vol) at 37°C (24 hr) and later analyzed spectrophotometrically for hemoglobin content at 418 nm. Samples of the solubilized material were also taken for determination of cell protein by the methods of Lowry et al. (13). The value for epithelial cell protein in each sample was corrected for concomitant erythrocyte protein from a standard curve of hemoglobin absorbance /mg erythrocyte protein prepared in each experiment.

Preparation of Fluorescein-Labeled Con A

Fluorescein isothiocyanate (FITC; Calbiochem, San Diego, Calif.) was conjugated with Con A (grade A, Calbiochem) in the ratio 2 FITC/1 Con A, at 4°C by gentle shaking

for 24 hr in normal Ringer's solution (pH 7.4). FITC-Con A was then purified by chromatography on Sephadex G-25.

Measurement of FITC-Con A Binding to Isolated Epithelial Cells

Epithelial cells were suspended in 5 ml normal Ringer's solution in polyethylene tubes (Falcon Plastics, Oxnard, Calif.) and stirred with a fine stream of O_2. Fifteen min of incubation in the presence or absence of added reagents immediately preceded incubation with 50 μg/ml FITC-Con A in normal Ringer's solution for 3 min. The cells were then washed twice by centrifugation at 400 × g for 3 min in a Sorvall RC2-B centrifuge. The sediment was suspended in 0.1% Triton/0.1 N NaOH and incubated at 37°C for 24 hr. The fluorescence due to bound FITC-Con A was measured in aliquots of the solubilized sediment by means of an Aminco-Bowman spectrophotofluorometer (490 nm excitation; 520 nm fluorescence).

Reagents

The concentrations of hormones to which the epithelial cells were exposed in the various experiments were as follows: ADH (Parke-Davis), 100 mU/ml suspension medium; and angiotensin II (grade A, Calbiochem), 0.5 μg/ml medium. In several experiments, the specificity of Con A binding and Con A-mediated hemadsorption to epithelial cells was tested following a 15-min incubation period with 0.1 M α-methyl-D-mannoside (Sigma), a specific hapten inhibitor of Con A binding (cf. Inbar and Sachs [14]).

RESULTS

Effects of Con A Concentration on Hemadsorption to Epithelial Cells

Effects of Con A concentration on hemadsorption to epithelial cells isolated from bullfrog urinary bladder are shown in Fig. 1. Hemagglutination in all subsequent experiments was conducted after incubation of cells with 50 μg Con A/ml suspension medium, a concentration maximal for Con A binding (see below) and submaximal for Con A-mediated hemadsorption. Addition of 50 μg Con A/ml in the presence of 0.1 M α-methyl-D-mannoside reduced subsequent lectin-mediated hemadsorption to 19 ± 7% of controls ($P < 0.01$, $n = 3$), thus demonstrating the specificity of the assay.

Effects of ADH Treatment and Incubation Time with Erythrocytes on Con A-Mediated Hemadsorption to Epithelial Cells

Hemadsorption to ADH-treated or untreated epithelial cells isolated from bullfrog urinary bladder was investigated at various times of incubation with homologous erythrocytes (Table I). In untreated cells, there was no significant variation in hemagglutinability with increasing time of incubation from 3 to 15 min at 22°C. Cells exposed to 100 mU ADH · ml^{-1} for 15 min at 22°C adsorbed significantly greater numbers of erythrocytes to 260%, 280%, and 272% of controls, respectively, at 3, 7, and 15 min of incubation with red blood cells.

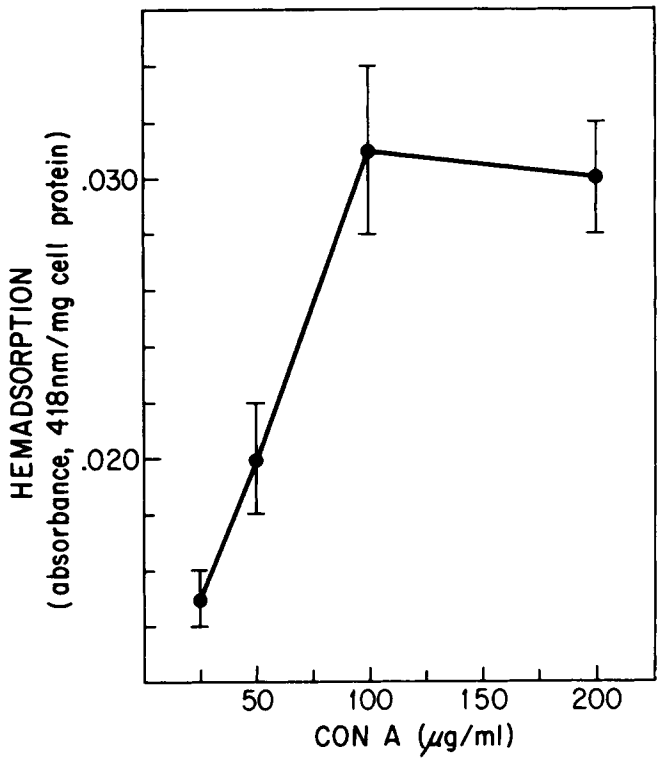

Fig. 1. Effects of Con A concentration on hemadsorption to epithelial cells isolated from bullfrog urinary bladder. The absorbance of amphibian erythrocyte hemoglobin associated with filtered/solubilized epithelial cells exposed to 25, 50, 100, or 200 μg. Con A·ml^{-1} (3 min at 22°C) was measured at 418 nm on a Gilford spectrophotometer. The result was then expressed relative to mg epithelial cell protein. Each point represents the mean ± SEM of at least three independent experiments.

Effects of Temperature and ADH Treatment on Con A-Mediated Hemadsorption to Epithelial Cells

The effect of variations in temperature and ADH treatment on Con A-mediated hemadsorption to bullfrog epithelial cells is presented in Fig. 2. Quantitative alterations in hemadsorption occurred above and below 22°C, the mean body temperature in these amphibians. Reduction of temperature to 4°C depressed ($P < 0.05$), while elevation of temperature to 37°C enhanced hemadsorption ($P < 0.05$) as compared with that observed at 22°C.

As above, ADH elicited a marked elevation of Con A-mediated hemagglutination at 22°C to 280% of the control value ($P < 0.001$). Likewise, in epithelial cells isolated from the urinary bladder of the toad and incubated at 22°C, ADH enhanced hemadsorption to 280% of the control value ($P < 0.001$) in three independent experiments (not shown).

Con A-mediated hemagglutination of bullfrog epithelial cells at either 4°C or 37°C was not influenced by ADH treatment ($P > 0.1$; Fig. 2). Hemadsorption at 4°C following

TABLE I. Effects of ADH Treatment and Incubation Time with Erythrocytes on Con A-Mediated Hemadsorption to Epithelial Cells Isolated from Bullfrog Urinary Bladder

Group	Incubation time (min)	Con A-mediated hemadsorption[1] (absorbance · mg protein^{-1})
Control	3	0.020 ± 0.001 (3)
100 mU ADH · ml^{-1}	3	0.052 ± 0.004 (3)[2]
Control	7	0.021 ± 0.004 (3)
100 mU ADH · ml^{-1}	7	0.059 ± 0.005 (3)[2]
Control	15	0.022 ± 0.002 (3)
100 mU ADH · ml^{-1}	15	0.060 ± 0.010 (3)[3]

The absorbance of bullfrog erythrocyte hemoglobin associated with epithelial cells exposed to 50 μg Con A · ml^{-1} (3 min at 22°C) was measured as described in the text. Cells were incubated in the absence (control) or presence of 100 mU ADH · ml^{-1} for 15 min (22°C) before addition of lectin.

[1] Values presented as mean ± SEM with the number of estimates in parentheses.
[2] Value significantly different from control at $P < 0.001$.
[3] Value significantly different from control at $P < 0.01$.

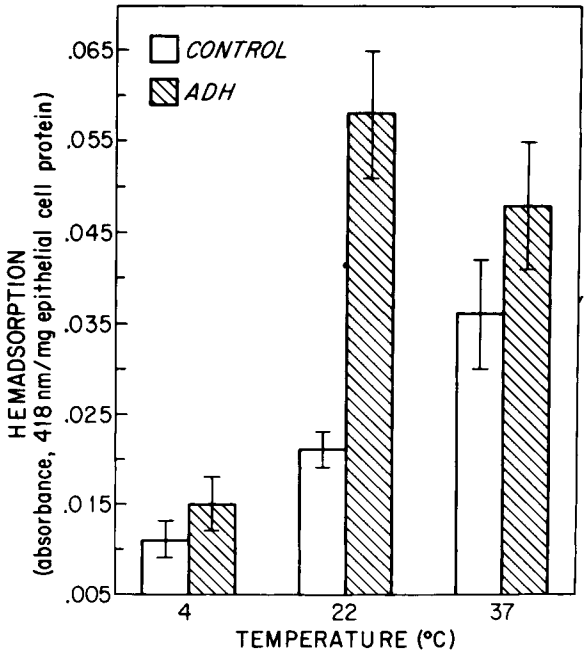

Fig. 2. The effects of temperature and ADH treatment on Con A-mediated hemadsorption to bullfrog epithelial cells. Cells were exposed to 50 μg Con A · ml^{-1} (3 min), washed, incubated with isologous erythrocytes (7 min) at 4°, 22°, and 37°C, washed again, and finally solubilized. Initially, cells were exposed to 100 mU ADH · ml^{-1} or equivalent volumes of control saline at 22°C followed by 15 min of incubation at the temperatures indicated on the abscissa before exposure to Con A.

ADH treatment was below that observed with untreated cells at 22°C ($P < 0.05$). Although hemadsorption at 37°C to hormone-treated cells was well above that observed in untreated cells assayed at 22°C ($P < 0.05$), it was not significantly different from hemadsorption at 22°C to cells treated with ADH. The apparent lack of influence of ADH treatment on hemadsorption to cells at 37°C as compared to that of untreated cells can be attributed to enhanced hemadsorption to the untreated cells with elevation of temperature from 22° to 37°C.

Effects of Con A Concentration and Time on FITC-Con A Binding to Epithelial Cells

Epithelial cells isolated from bullfrog bladders were exposed to various concentrations of Con A as shown in Table II. The cells bound lectin maximally at a concentration of 50 μg Con A · ml^{-1}. Variations in the time of incubation of cells with this latter Con A concentration indicate that lectin binding became maximal at 3 min of incubation (see Table II). Addition of 50 μg Con A · ml^{-1} to cells incubated with 0.1 M α-methyl-D-mannoside reduced binding to 17 ± 10% of controls ($P < 0.001$, $n = 3$), demonstrating the specificity of FITC-Con A binding.

Effects of Temperature and ADH Treatment on Binding of FITC-Con A to Epithelial Cells

Bullfrog epithelial cells were maintained for 15 min at the selected test temperature before the start of the binding assay. The effects of temperature and ADH treatment on binding of FITC-Con A to the epithelial cells are presented in Fig. 3. Binding levels at either 22° or 37°C to cells treated or not treated with hormone were not significantly different from each other. However, binding at 4°C to cells incubated in the presence or absence of ADH was significantly depressed as compared with binding to cells incubated at the higher temperatures ($P < 0.001$).

These data, therefore, indicate that the increased agglutination observed with untreated cells at 37°C as compared with 22°C, and at 22°C as a result of ADH treatment are not due to differences in the amount of Con A bound. The limited hemadsorption at 4°C, however, does correspond to a significantly lower binding of the lectin.

Effect of Angiotensin II Treatment on Con A-Mediated Hemadsorption and Binding of Con A to Epithelial Cells

Since angiotensin is known to promote minor enhancement of osmotic water permeability in amphibian bladder (15), the effects of 0.5 μg angiotensin II · ml^{-1}, a concentration approximately equimolar with 100 mU ADH · ml^{-1}, on Con A binding and Con A-mediated hemadsorption to epithelial cells were investigated. As in the case of ADH, angiotensin failed to alter FITC-Con A binding when compared to untreated cells (Table III). However, angiotensin elicited a small but significant ($P < 0.05$) increment in lectin-mediated hemadsorption to epithelial cells from bullfrog bladder. In this respect, angiotensin, which is also an octapeptide hormone, appears to elicit a cell surface effect similar to that of ADH.

TABLE II. Effects of Con A Concentration and Time on FITC-Con A Binding to Epithelial Cells Isolated from Bullfrog Bladder

Con A concentration ($\mu g \cdot ml^{-1}$)	Incubation time (min)	Net fluorescence FITC-Con A · mg protein^{-1} (Mean ± SEM [n])
15	3	3.7 ± 0.3 (3)
30	3	7.4 ± 0.1 (3)*
50	1	10.6 (2)
50	3	12.4 ± 0.8 (3)*
50	5	12.4 (2)
50	10	12.2 (2)
100	3	13.2 ± 0.4 (3)

Cell were exposed to FITC-Con A at various concentrations of lectin as indicated at 22°C. At a concentration of 50 μg Con A · ml^{-1}, the level at which maximal lectin binding is detected, the influence of incubation time on binding is also shown. A spectrophotofluorometric value of 6 net fluorescence (arbitrary units) is equivalent to approximately 0.10 μg of Con A.

*Value significantly different from lower Con A concentration at $P < 0.01$.

TABLE III. Effects of Angiotensin II Treatment on Con A-Mediated Hemadsorption and Binding of Con A to Epithelial Cells Isolated from Bullfrog Urinary Bladder

Group	FITC-Con A binding (net fluorescence · mg protein^{-1})	Con A-mediated hemadsorption (absorbance · mg protein^{-1})
Control	12.5 ± 0.3 (3)	0.022 ± 0.003 (5)
0.5 μg Angiotensin II · ml^{-1}	11.8 ± 0.3 (3)	0.030 ± 0.002 (5)

Bullfrog epithelial cells were incubated at 22°C in the absence (control) and presence of angiotensin for 15 min. Con A binding and Con A-mediated hemadsorption to epithelial cells were then determined, as described in the text, at 22°C. All values are presented as mean ± SEM with the number of estimates in parentheses.

DISCUSSION

The effects of α-methyl-D-mannoside reported here demonstrate that Con A binds with specificity to membranes of epithelial cells isolated from amphibian urinary bladders. Furthermore, this lectin also mediates adsorption of homologous erythrocytes to these cells. The observed effects of temperature on both Con A binding and Con A-mediated hemagglutination are similar to those reported for mouse fibroblasts (16). At 4°C, both binding and hemadsorption are reduced from the levels measured at higher temperatures. As revealed by electron spin resonance spectroscopy, the membrane lipids of mammalian cells undergo a change in physical state as temperature is decreased from 37°C to 15°C (17). These alterations in physical state can restrict the lateral mobility of antigens that penetrate the membrane hydrocarbon phase (18) as well as the partition of solutes into the

membrane (19–21). Since Con A-mediated agglutination appears to require lateral mobility of lectin receptors in the plane of the membrane (5), the inhibition of agglutination at low temperature in the present and previous studies (16, 22) indicates that the relative fluidity of membrane lipids may influence the availability or orientation of Con A receptors. The observed increment in lectin binding at higher temperatures (Fig. 3), however, may also be attributable, in part, to enhancement of pinocytotic activity.

The absence of an ADH effect on hemagglutination of epithelial cells at 4°C may also be related to physicochemical or structural factors. The effect of ADH on osmotic water permeability of toad urinary bladder is markedly delayed at low temperatures (23). By the same token, incubation at 4°C is also known to disrupt microtubule structure in the intact cell (see Olmstead and Borisy [24]). This latter observation may be pertinent to recent reports which suggest that microtubules and microfilaments play an important role in the cellular action of ADH (see Taylor et al. [25]).

Increments in Con A-mediated hemadsorption as a result of temperature elevation, ADH, and angiotensin cannot be ascribed to changes of a comparable order in Con A binding. ADH treatment elicited a marked increase in hemadsorption at 22°C with respect to that of untreated cells. This effect at 37°C in hormone-treated cells is likewise significantly greater than in untreated cells at 22°C, but not significantly different from untreated cells incubated at 37°C. This dichotomy is attributable to a disproportionate augmentation in the hemagglutinability of untreated cells following elevation of the incubation temperature from 22°C to 37°C. This observation is paralleled by marked increments in osmotic water permeability across artificial lipid membranes and progressive reductions in the transport-enhancing action of ADH with elevation in temperature from 20° to 37°C (see Fig. 3 in reference 26).

In the absence of a corresponding degree of augmentation in Con A binding, the enhanced hemadsorption of epithelial cells in response to temperature and hormones may result from an increase in the motional freedom of membrane molecules in both cases. Several additional factors which may contribute to such an enhancement of cellular agglutinability have been considered in other independent investigations (cf. references 6, 8, 16). It is unlikely that the early increase in the hemagglutinability of bladder epithelial cells is dependent on increments in membrane surface area since Grantham (27) has previously reported that ADH does not elicit any detectable change in size of mucosal cells in the hormone-responsive collecting duct of the rabbit. Chevalier et al. (28), however, have recently found that treatment of frog urinary bladder epithelium with oxytocin elicits a redistribution of membrane particles (i.e. proteins) associated exclusively with the apical membrane. In addition, the mobility of spin label and fluorescent probes in the lipid phase of several cell types is known to increase in response to addition of the appropriate polypeptide hormone (i.e. angiotensin II [29], thyroid-stimulating hormone, adrenocorticotrophic hormone [30], and growth hormone [31, 32]) or to temperature elevation (30). Similarly, evidence obtained from lipophilic probes of amphibian bladder membranes indicates that ADH acts to enhance the disorder or fluidity of membrane lipids (33). Such increments in membrane fluidity attributable to hormone or to temperature elevation may contribute to a redistribution and aggregation of lectin-binding proteins detected in the present studies.

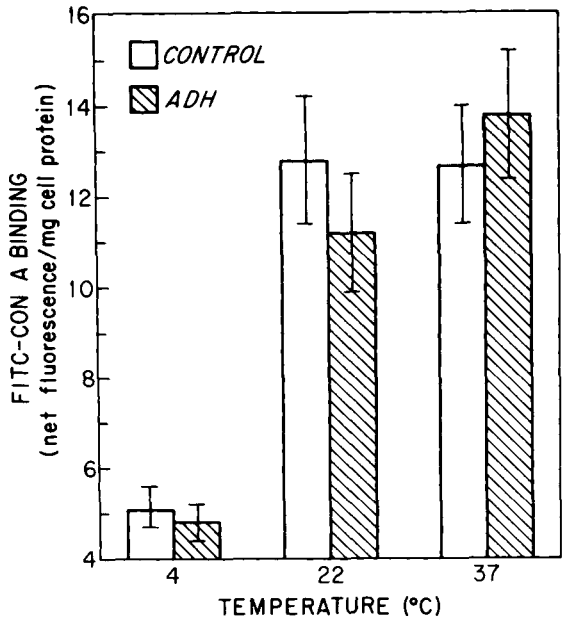

Fig. 3. Effects of temperature and ADH treatment on FITC-Con A binding to bullfrog epithelial cells. Cells were exposed to FITC-Con A for 3 min, following a 15 min incubation in the presence and absence of ADH (see Fig. 2), at the temperature indicated on the abscissa.

Allison (34) has presented indirect evidence to suggest that agents which promote the aggregation of membrane glycoproteins may thereby generate new hydrophilic pathways for enhanced ion and nonelectrolyte movement. This permeability pattern is characteristic of the apical membrane action of ADH on granular epithelial cells from amphibian bladder (33, 35). The apparent analogy here indicates that further investigations should be undertaken to determine whether hormone-induced changes in lectin-mediated cellular agglutinability, membrane fluidity, surface protein distribution, and transport are causally related. The alteration of transport and surface properties of cell membranes attributable to ADH may also occur secondary to the migration to apical membrane of intracellular secretion granules (36), which may correspond to lysosomes (37), with concomitant extracellular release of lysosomal enzymes, including cathepsin B1 (10, 38). Regardless of the precise mode of action of antidiuretic and other polypeptide hormones, the procedures described here should prove useful in future investigations of the effects of these agonists on the surface properties of isolated target cells.

ACKNOWLEDGMENTS

This study was aided by research grants MH 6415, HD 4354, and FR 7009 from the United States Public Health Service, GB 36177 from the National Science Foundation, and by general research funds of the University of California.

We gratefully acknowledge the advice of Stephen S. Lippman in the preparation of the FITC-Con A.

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