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Stimulation of Lymphocyte Receptor Capping by the Ionophore Monensin

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Summary. The carboxylic ionophore monensin has a biphasic effect on antibody-induced Thy-1 cap formation. At higher concentrations, 5×10^{-6}–5×10^{-5} M monensin causes a significant inhibition of receptor capping similar to that previously found with the Ca^{2+} selective ionophore A23187. At lower concentrations, 5×10^{-8}–5×10^{-7} M capping is stimulated. It is concluded that capping at lower ionophore concentrations is a specific response to the ability of monensin to induce a rise in intracellular Na^+, which indirectly elevates intracellular Ca^{2+} activity. This in turn activates the contractile machinery required for the aggregation of surface receptors into capped structures. At higher concentrations monensin acts as a nonspecific detergent, which causes detrimental structural alterations in some of the membrane components involved in the capping process.

Key Words receptor · capping · monensin · Na^+ · Ca^{2+} · contractile machinery

Introduction

It is well known that a large number of multivalent ligands (e.g., antibodies raised against cell surface antigens), upon binding to their specific receptor molecules in the plasma membrane of lymphoid cells, induce a redistribution of the membrane receptors into small clusters (so-called *patches*). These patches can then aggregate via an energy-dependent process at one pole of the cell to form *caps* on the cell surface (Taylor, Duffus, Kaff & dePetris, 1971; Schreiner & Unanue, 1976*a*). Previously we had demonstrated a close association between intracellular contractile proteins and surface cap structure (Bourguignon & Singer, 1977; Bourguignon, Takuyasu & Singer, 1978, Bourguignon, 1980; & Rozek, 1980; Butman, Bourguignon & Bourguignon, 1980; Bourguignon, Nagpal & Hsing, 1981). Subsequently a transmembrane interaction theory was suggested in which ligand (antibody)-receptor complexes are proposed to function in the attachment of certain membrane protein molecules to acto-myosin filaments and thereby induce the aggregation of certain surface receptors into a cap structure via a sliding filament mechanism analogous to that of muscle contraction (Bourguignon & Singer, 1977). Ca^{2+} is known to be involved in a variety of membrane-related cytoskeletal cellular activities, including exocytosis, phagocytosis, membrane deformation and receptor capping (Huxley, 1969; Manery, 1969; Shibata et al., 1972; Pollard & Weihing, 1974; Durham, 1974; Taylor, 1975; Schreiner & Unanue, 1976*a*; Bourguignon & Balazovich, 1980; Bourguignon & Kerrick, 1982). However, the question of whether monovalent Na^+ plays a role in membrane/cytoskeletal activity such as receptor capping has not yet been considered. Since Na^+ has been found to interact with Ca^{2+} in many biological systems, it would clearly be of interest to allow various amounts of Na^+ to enter the cytoplasm and then examine the cells for possible effects on lymphocyte capping. The carboxylic ionophore monensin, is ideal for this purpose since it promotes a one-for-one exchange of intracellular K^+ for extracellular Na^+ and has a negligible ability to transport Ca^{2+} directly (Pressman, 1976).

Materials and Methods

Cells

The mouse T-lymphoma cell line, AKR/J lymphoma line BW5147 (gift from Dr. R. Hyman, Salk Institute), was grown in Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated horse serum (Gibco Laboratories, Grand Island, N.Y.) at 37 °C in 5% CO_2 95% air.

Effect of Monensin on Lymphocyte Receptor Capping

Cultured mouse T-lymphoma cells were treated with various concentrations of monensin ($5 \times 10^{-5} - 5 \times 10^{-9}$ M) in RPMI

1640 medium. Control samples were incubated with RPMI 1640 medium alone in the absence of monensin at room temperature for 30 min, followed by the addition of antibodies. Cells, pre-incubated either with or without monensin, were labeled with monoclonal rat antibodies against Thy-1 for 15 min at 37 °C and rabbit anti-rat immunoglobulin for another 15 min at 37 °C. The cells were subsequently fixed with 2% paraformaldehyde and stained with fluorescein-conjugated goat anti-rabbit immunoglobulin. Fluorescent-labeled samples were examined under a Zeiss photomicroscope using a 40 × oil immersion lens with epi-illumination.

Results and Discussion

Our data show that treatment of the mouse T-lymphoma cells with monensin at the concentrations between 5×10^{-5} M and 5×10^{-6} M causes a significant inhibition of antibody-induced Thy-1 cap formation (Fig. 1). Specifically, capping is inhibited 46% by treatment at 5×10^{-6} M and almost 90% by 5×10^{-5} M (Fig. 1). Cells treated with these concentrations of monensin preferentially display patch-like structures which fail to aggregate into caps (Fig. 2*a*). In contrast to these findings, we have observed a *stimulatory* effect on lymphocyte capping by monensin at concentrations less than 5×10^{-7} M , showing the most pronounced effect at 5×10^{-8} M and little effect at the dose below 5×10^{-9} M (Fig. 1). In cells treated with low levels of monensin, the surface receptors are aggregated into tight cap structures (Fig. 2*b*).

The biphasic response of antibody-mediated lymphocyte receptor distribution to monensin can be interpreted in the following manner. A rise in intracellular Ca^{2+} activity may serve to promote the association of the acto-myosin assemblies with the lymphocyte membrane as proposed in the red cell membrane-spectrin system (Nicolson, Marchesi & Singer, 1971); alternatively Ca^{2+} may bind to calmodulin or other regulatory molecules necessary for activation of contractile elements involved in receptor capping (Bourguignon & Balazovich, 1980). Low doses of monensin are known to increase intracellular Na^{+} (Pressman & Fahim, 1982), which would raise intracellular Ca^{2+} activity, either by releasing *intracellularly* sequestered Ca^{2+} or exchanging with *extracellular* Ca^{2+} (van Breemen, Aaronson & Loutzenhizer, 1979). Monensin-promoted capping then can be explained by the indirect rise in intracellular Ca^{2+} activity induced by the ionophore. A number of cells thought to be intrinsically activatable by Ca^{2+} are stimulated by low levels of monensin (10^{-7}–10^{-8} M, including cultured adrenal chromaffin (Suchard, Lattanzio, Rubin & Pressman, 1982) and heart cells (Howard, Brumshwig & Pressman, 1976).

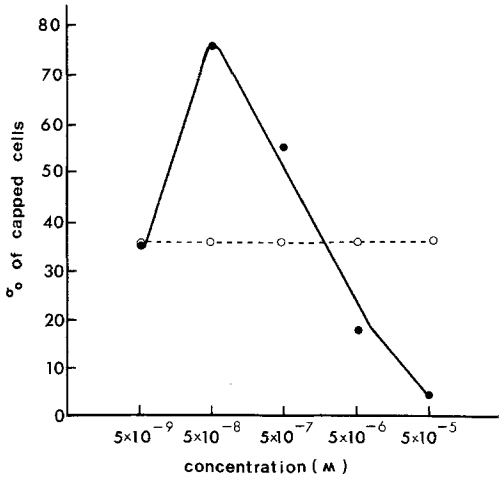

Fig. 1. Effect of Monensin on Receptor Capping. Cultured mouse T-lymphoma cells were first treated with various concentrations of monensin (5×10^{-5}–5×10^{-9} M) in RPMI 1640 medium. Control samples were incubated with RPMI 1640 medium alone in the absence of monensin at room temperature for 30 min, followed by the addition of antibodies. Cells, pre-incubated either with or without monensin, were labeled with monoclonal rat antibodies against Thy-1 for 15 min at 37 °C and rabbit anti-rat immunoglobulin for another 15 min at 37 °C. The cells were subsequently fixed with 2% paraformaldehyde and stained with fluorescein-conjugated goat anti-rabbit immunoglobulin. Fluorescent-labeled samples were examined under a Zeiss photomicroscope using a 40 × oil immersion lens with epi-illumination.

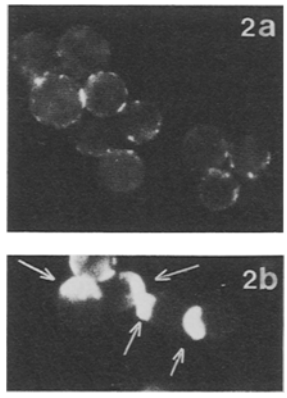

Fig. 2. (*a*): Patch-like structures of Thy-1 receptors, when T-lymphoma cells were treated with antibodies in the presence of 10^{-5}–10^{-6} M monensin as described in Fig. 1. (1200 ×) (b) Cap formation of Thy-1 receptors (↑), when T-lymphoma cells were treated with antibodies in the presence of 10^{-8} M monensin as described in Fig. 1. (1200 ×)

At higher levels ionophores may act as detergents, owing to their amphipathic structure. Thus in the range 10^{-5} –10^{-6} M monensin may damage some elements of the cell membrane required for capping, halting the distribution of receptors at patching stage. Even the value at 5×10^{-7} M monensin (Fig. 1) presumably represents partial inhibi-

tion since it is significantly lower than the maximal capping found at 5×10^{-8} M monensin. Such membrane-related damage, at higher concentrations of ionophores, has been associated with the release of lactic dehydrogenase from chromaffin (Suchard et al., 1982) and pancreatic cells (Singh, 1980). Other inhibitory processes attributed to long term exposure of cultured cells to monensin (10^{-5}–10^{-6} M) include the inhibition of the formation of Golgi-related vacuoles (Ledger, Uchida & Tanzer, 1981) and recycling of lipoprotein receptors of the plasma membrane (Basu, Goldstein, Anderson & Brown, 1981).

The Ca^{2+} selective ionophore, A23187 (Reed & Lardy, 1972) has been reported to *inhibit* receptor capping in B-lymphocytes; however, it was used at relatively high doses (10^{-6}–10^{-7} M) (Schreiner & Unanue, 1976*a*, *b*). Thus the inhibition of capping by A23187 may have been due to nonspecific detergency properties of the ionophore rather than a rise in intracellular Ca^{2+} activity. The ability of low levels of monensin to *stimulate* capping which we observe may be a more valid attribution of a rise in intracellular Ca^{2+} activity than the capping *inhibition* produced by the high levels of A23187 (Schreiner & Unanue, 1976*a*, *b*). As a corollary of our results, we would advise caution in interpreting results whenever high levels (10^{-5}–10^{-7} M) of ionophores are applied to cells, as is commonly the case when A23187 is employed to trigger cell activation by addition of intracellular Ca^{2+}.

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