Role of Calcium in Gamma Interferon Induction: Inhibition by Calcium Entry Blockers

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Mitogen-induced gamma interferon production by human lymphoid cell cultures was studied in the presence of calcium entry blockers. A dose-dependent inhibition was found in the presence of drug concentrations down to 10^{-5} M. This finding shows that calcium flow through lymphocyte membranes after oxidation of membrane-bound galactose residues is also critical for triggering interferon production.

Mitogenic, as well as antigenic, stimulation of gamma interferon (gamma-IFN) production by human lymphocytes occurs through oxidation of galactose residues on the cell membrane (1a, 3, 4). A role for calcium was suggested by the finding that gamma-IFN production was stimulated by the calcium ionophore A23187 (2) and that IFN induction by mitogens (3), antigens, and oxidizing agents (1a) did not occur in the presence of the chelating agent ethylene glycol-bis(β-aminopropyl ether)-N,N,N-tetraacetic acid. Moreover, IFN production was also prevented when ethylene glycol-bis(β-aminopropyl ether)-N,N,N-tetraacetic acid was added to the cultures after completion of the oxidative events on the cell membrane (1a). Taken together, these findings suggest that activation of a calcium flow through the lymphocyte membrane represents a subsequent specific step, after oxidation of membrane-bound galactose residues, in triggering IFN production. However, it would also be possible, although unlikely, that calcium depletion may block IFN induction nonspecifically by affecting some other cell function. To rule out this possibility, we studied mitogen-induced gamma-IFN production in the presence of calcium antagonists whose action has been shown to be highly selective in blocking calcium entry through specific channels (1, 5, 6, 9, 10).

Lymphoid cell cultures (10^6 cells per ml) were established as previously described by Ficoc-Hypaque gradient sedimentation of peripheral blood from healthy adult donors (4). RPMI 1640 medium supplemented with 10% fetal calf serum was used as culture medium. Nitrendipine, nisoldipine and nimodipine were obtained from Miles Laboratories, Inc., Elhart, Ind. Verapamil chloride was kindly provided by Knoll Pharmaceutical Co., Whippany, N.J., and diltiazem was provided by Marion Laboratories, Kansas City, Kans. Trifluoperazine dihydrochloride, procaine chloroformate F.U., and MnCl₂ were purchased from commercial sources. Lymphoid cell cultures were treated with the T-cell mitogen staphylococcal enterotoxin B (0.25 μg/ml; Sigma Chemical Co., St. Louis, Mo.) in the presence of absence of the compounds added at concentrations varying from 10^{-3} to 10^{-6} M. The cultures treated with MnCl₂ were supplemented with HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid) buffer (pH 7.2).

IFN production was determined after 18 h of incubation at 37°C. IFN titrations were performed with human WISH cell cultures; the activity was measured by inhibition of Sindbis virus hemagglutinin yield as previously described (4). Toxicity of the compounds was evaluated by the trypsin blue uptake criterion, by lack of inhibition of protein synthesis in the treated lymphocytes (after a 1-h pulse with 0.25 μCi of [35S]methionine), and by lack of inhibition of alpha-IFN production after stimulation with Newcastle disease virus.

The first blocker of calcium channels used was nitrendipine. The results of a representative experiment are shown in Fig. 1 in which a dose-response curve of nitrendipine is reported. It may be seen that IFN induction by staphylococcal enterotoxin B was completely suppressed in the presence of the compound up to 10^{-4} M, and it was substantially and significantly inhibited up to 10^{-5} M concentration. On the contrary, procaine, a blocker of sodium channels (8), did not significantly affect IFN induction by staphylococcal enterotoxin B. Substantially identical results were obtained by using lymphocytes from different donors and other mitogens as the inducers (galactose oxidase, concanavalin A). Simultaneous control experiments showed that the concentrations

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FIG. 1. Effect of various concentrations of nitrendipine and procaine on gamma-IFN production by mitogen-stimulated human lymphoid cell cultures.

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of nisoldipine which inhibited gamma-IFN production did not have any effect on cell viability (>95% survival), on protein synthesis (no significant difference of [35S]methionine incorporation as compared to untreated controls), or on alpha-IFN production after induction with Newcastle disease virus.

The results of the experiments carried out with other calcium blockers are shown in Table 1.

It can be seen that all compounds tested show similar activity, with the exception of diltiazem, which has been reported to be a weaker antagonist of calcium (7). It can also be seen that a 24-h exposure of nisoldipine to light, a treatment which abolishes its effect on calcium, almost completely suppresses its inhibitory activity on IFN production.

Inhibition of gamma-IFN production by compounds which have been extensively characterized as specific blockers of calcium entry (1, 5-7, 9-11, 13) confirms the hypothesis that activation of a calcium flux through the lymphocytic membrane is indeed critical for mitogenic activation of gamma-IFN production (1a, 2). In fact (i) IFN production is inhibited in a dose-dependent manner; (ii) photoinactivation abolishes the IFN inhibitory activity; (iii) procaine, an inhibitor of sodium flux, does not affect IFN production; (iv) the inhibition of IFN production parallels the calcium blocking activity, as shown by diltiazem, a relatively weak calcium entry blocker which is also a weak inhibitor of IFN production. Additionally, the same effect is obtained with trifluoperazine dihydrochloride, an intracellular inhibitor of calmodulin function (12).

The inhibitory activity of most of the compounds studied ranged between 10^{-4} M and 10^{-3} M. Although these concentrations are higher than those which have been shown to be active in inhibiting contraction of rabbit aortic strip induced by potassium depolarization (11), they are not very different from those shown active in other experimental systems (13); additionally, to our knowledge, no dose-response curve has been so far reported for lymphocyte functions.

In our opinion, the findings reported in this paper are not only promising for providing a deeper insight into the regulation of lymphocyte activation and gamma-IFN production, but they may also have practical implications; for instance, the possibility of using these compounds to prevent or treat lymphokine-mediated hypersensitivity reactions is now under study.

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LITERATURE CITED