Use of fluoride ion as a probe for the guanine nucleotide-binding protein involved in the phosphoinositide-dependent neutrophil transduction pathway

Colette F. Strnad*, Janice E. Parente and Kenneth Wong

Departments of Pharmacology and Medicine, University of Alberta, Edmonton, Alberta, T6G 2G3 Canada

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Fluoride activation of neutrophils was found to be associated with phosphoinositide turnover, as monitored by the time-dependent accumulation of inositol phosphates. Unlike phosphoinositide turnover induced by the chemotactic peptide, formylmethionylleucylphenylalanine, that induced by fluoride was not inhibited by pretreatment with pertussis toxin. The translocation of protein kinase C activity from the cytosolic to the membrane compartment was also observed in fluoride-stimulated cells. We have proposed that the mode of action of this halide ion involves interaction with a GTP-binding protein which serves as an intermediary unit between the receptors for inflammatory sttimuli and the phosphoinositide-specific phosphodiesterase.

Guanine nucleotide-binding protein *Neutrophil* Fl^-

1. INTRODUCTION

Although the ability of fluoride to activate superoxide production [1], calcium mobilization [2] and arachidonic acid release [3] in the neutrophil has been recognized for some time, few attempts have been made to discern the molecular mechanisms of action of this ion as an inflammatory stimulus. In view of fluoride's efficacy as an activator of both the N_s and N_i guanine nucleotide-binding proteins of the adenylate cyclase system [4], we have examined the possibility that fluoride-induced inflammatory responses may be occurring at the level of a G-protein which has been suggested to serve as an intermediary unit between the receptors for certain inflammatory signals and the phosphoinositide-specific phosphodiesterase [5–7]. Were this the case, fluoride would be expected to mimic the action of 'calcium-mobilizing' receptor stimuli in activating the phosphodiesterase with the resultant degradation of phosphoinositides and redistribution of protein kinase C activity. The following data indicate that fluoride is an effective stimulant for both of these events.

* Correspondence address: 9-112 Clinical Sciences Building, University of Alberta, Edmonton, Alberta T6G 2G3, Canada

2. EXPERIMENTAL

Neutrophils were isolated from the venous blood of healthy volunteers as described [8]. Superoxide radical production was monitored at 550 nm by the increase in absorbance of the indicator horse heart ferricytochrome *c* [8].

Inositol phosphates were extracted from the neutrophils by a variation of the procedure of Bradford and Rubin [9]. Prior to stimulation with the reagents in question, cells were preincubated for 10 min with 10 mM LiCl. After fixed time in-

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tervals of exposure to reagents, reactions were stopped by the addition of 5% trichloroacetic acid. Ether washed acid extracts were subjected to anion-exchange columns on Dowex resin (AG1-X8, formate form, Bio-Rad) as described by Berridge [10].

For protein kinase C assays, neutrophils at a cell density of 20×10^6/ml were stimulated with reagents for fixed time intervals which were terminated by a sonication procedure followed by a 60 min centrifugation at $100000 \times g$. The supernatant was retained as the cytosolic fraction, while the pellet, representative of the membrane fraction, was resuspended in incubation buffer supplemented with 0.2% Triton X-100. The protein kinase C assay was based on the procedure of Kishimoto et al. [11].

3. RESULTS AND DISCUSSION

Stimulation of neutrophils with 18 mM NaF was observed to result in the accumulation of inositol 1-monophosphate (IP_1), inositol 1,4-bisphosphate (IP_2) and inositol 1,4,5-trisphosphate (IP_3), the water-soluble metabolites of phosphoinositide breakdown (fig.1). As is consistent with the superoxide production and calcium-mobilization aspects of fluoride-induced neutrophil activation, phosphoinositide turnover was characterized by a sustained duration of action. Owing to phosphatase-mediated inositol phosphate degradation, which tends to preclude the net accumulation of these metabolites during long term responses, 10 mM LiCl was added to the reaction mixture 10 min preceding the addition of stimulus to inhibit the breakdown of IP_1 by IP_1-phosphomonoesterase [12–14]. Due to the site of action of this inhibitor, the major inositol phosphate detected was IP_1. Because levels of IP_2 and IP_3 showed a fairly high degree of variability under such conditions, subsequent inhibitor studies focused on IP_1 formation.

As reported in [15,16], pertussis toxin inhibited the fMet-Leu-Phe-evoked phosphoinositide turnover response (fig.2), reinforcing the theory of G-protein involvement in the transduction pathway. fMet-Leu-Phe-induced phosphoinositide turnover differed from that elicited by fluoride in being of more rapid onset (approx. 15 s) and termination (1–2 min). Conversely, fluoride-

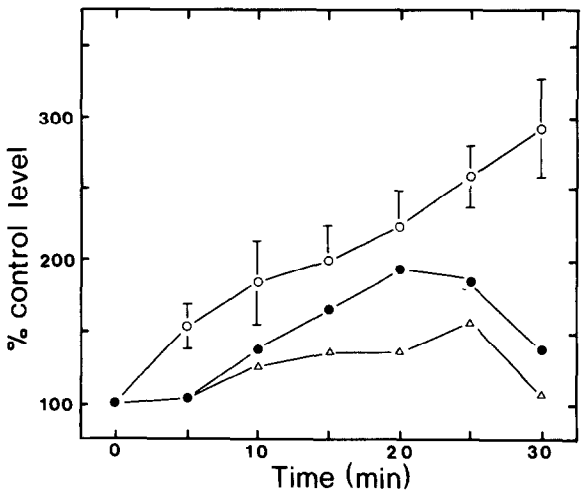

Fig.1. Time course of inositol phosphate accumulation in fluoride-activated neutrophils. Aliquots containing 2×10^7 cells, radiolabelled with [^{3}H]inositol, were activated with 18 mM NaF for the time periods shown. Results are expressed as percent of control value (i.e. levels in resting cells prior to NaF addition). The values for IP_1 (○) represent the mean ± SE for at least 6 independent experiments. The values for IP_2 (●) and IP_3 (△) represent the average of duplicate samples from a single experiment which is representative of at least 4 experiments. Control levels averaged 1122.5 dpm for IP_1, 469 dpm for IP_2, and 277 dpm for IP_3 per 2×10^7 cell sample.

activated IP_1 accumulation was resistant to inhibition by pertussis toxin, suggesting that ADP-ribosylation of the protein does not interfere with fluoride's action. Blackmore et al. [17] have reported that fluoride mediates phosphoinositide turnover in hepatocytes at the level of a guanine nucleotide-binding protein. Although this G-protein appears to be structurally distinct from its counterpart in the neutrophil in being insensitive to pertussis toxin inhibition in the presence of hormonal stimulation, it is interesting to speculate that the G-protein involved in the transduction of calcium-mobilizing stimuli may exhibit heterogeneity between tissue types.

Fluoride-induced IP_1 production also differed from that elicited by fMet-Leu-Phe in being resistant to inhibition by a 20 min preincubation with dibutyryl cAMP (not shown), a finding which corroborates our previous observation that fluoride-induced superoxide production is unaffected by

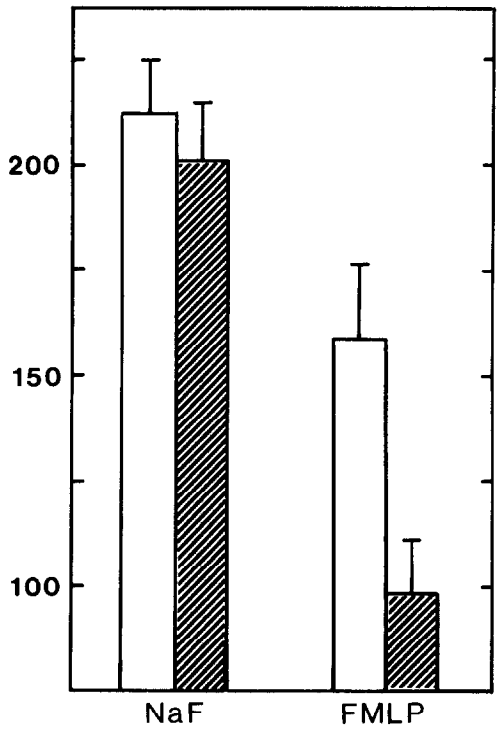

Fig.2. Effect of pertussis toxin on fluoride and fMet-Leu-Phe-activated IP_1 production. Radiolabelled cell suspensions were preincubated for 90 min in the presence (■) or absence (□) of 1 μg/ml pertussis toxin prior to challenge with 1 μM fMet-Leu-Phe or 18 mM NaF, for 60 s and 20 min time intervals, respectively. LiCl was added 10 min prior to fMet-Leu-Phe or NaF. Values represent the mean ± SE of at least 5 determinations.

adenylate cyclase agonists [18]. This finding suggests that cAMP-mediated inhibition of neutrophil activation is occurring either at the level of the G-protein in a manner which does not interfere with fluoride activation or else at the level of the chemotactic peptide receptor. It would thus appear that phospholipase C and other enzymes directly involved in the phosphoinositide turnover response are not the site of cAMP-mediated inhibition of signal transduction.

Inositol phosphate production in a stimulated cell system is associated with the concomitant generation of 1,2-diacylglycerol, a product linked with the activation protein kinase C [19]. Activation of neutrophils with 18 mM NaF was characterized by the loss of protein kinase C from the cytosolic fraction and a concomitant increase in membrane-associated protein kinase C activity (fig.3). This translocation event was preceded by a lag interval of 5–10 min and displayed a prolonged duration, a time course corresponding to that of the associated superoxide generation. Protein kinase C translocation appeared to be most pronounced in cells incubated with fluoride for 15 min, after which a slow return to baseline levels was noted. Notably, the point of maximal translocation corresponded to that at which the rate of fluoride-induced superoxide production was maximal (fig.3). Termination of the respiratory burst normally occurred 35–60 min after the addition of fluoride to cells, indicating that NADPH oxidase, the superoxide-generating complex, continued to turn over for some time after complete recovery of cytosolic protein kinase C activity.

The currently recognized G-proteins, N_s, N_i, N_o, and transducin, exist as trimers of α-, β- and γ-subunits (review [20]). In its resting state, the α-

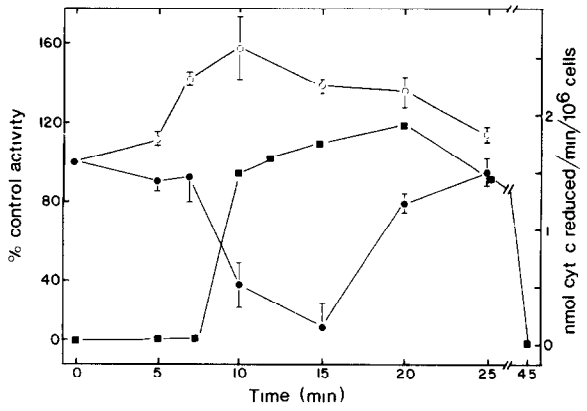

Fig.3. Time course of protein kinase C translocation and superoxide production rate in fluoride-activated neutrophils. Cell suspensions incubated with fluoride for fixed time intervals were assayed for protein kinase C activity in the cytosolic (●) and membrane (○) fractions. The values shown represent the mean ± SE of 6 experiments. The rate of superoxide production (■), as determined by the rate of cytochrome *c* reduction, was determined at various time points on a continuous tracing curve. The values shown are based on a single experiment which is representative of at least 10 experiments.

subunit is postulated to bind a GDP moiety, which it exchanges for GTP upon interaction of the G-protein with an activated hormone receptor complex. Binding of GTP is associated with the dissociation of the G-protein to release N_α-GTP which, in the phosphoinositide system, has been envisioned to interact with the phosphoinositide-specific phosphodiesterase, so reducing the calcium requirement of the latter enzyme to physiological ranges [7]. Deactivation presumably occurs upon hydrolysis of GTP by the intrinsic GTPase activity of the α-subunit [20]. Fluoride has been reported to induce the dissociation of chromatographically resolved G-proteins into the α monomer and β-γ dimer in a GTP-independent manner which is not readily reversible [21,22]. It has been suggested that fluoride complexes with aluminum contaminants to form the active species AlF_4 which serves to activate the G-protein by virtue of its ability to mimic the γ-phosphate of GTP at the GTP-binding site on the G-protein [23]. Common glassware is known to release sufficient quantitites of aluminum to account for G-protein inactivation in the presence of NaF [24]. In our studies, introduction of micromolar amounts of $AlCl_3$ to incubation mixtures had no additional effect upon cellular responses even at submaximal fluoride concentrations and, in fact, resulted in nonspecific inhibitory effects. For this reason, supplementation of assay mixtures with aluminum salts was not indicated.

Our present results are consistent with the premise that fluoride-induced neutrophil activation is occurring at the level of a guanine nucleotide-binding protein serving as a transducing unit between the receptors for inflammatory stimuli and the phosphoinositide-specific phosphodiesterase. Although it is regrettable that no pharmacological activator has yet been identified with the ability to distinguish selectively between various forms of the G-proteins, the use of fluoride as a probe for the G-protein involved in neutrophil activation provides a useful tool with which to stimulate this transducing unit without resorting to the potentially artifactual membrane preparations or cell permeabilization procedures necessary for G-protein activation with GTP or its analogues.

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REFERENCES

[1] Curnutte, J.T., Babior, B.M. and Karnovsky, M.L. (1979) J. Clin. Invest. 63, 637–647.
[2] Strnad, C.F. and Wong, K. (1985) Biochem. Biophys. Res. Commun. 133, 161–167.
[3] Bokoch, G.M. and Gilman, A.G. (1984) Cell 39, 301–308.
[4] Smigel, M., Katada, T., Northup, J.K., Bokoch, G.M., Ui, M. and Gilman, A.G. (1984) in: Advances in Cyclic Nucleotide and Protein Phosphorylation Research (Greengard, P. et al. eds) pp.1–8, Raven, New York.
[5] Cockcroft, S. and Gomperts, B.D. (1985) Nature 314, 534–536.
[6] Barrowman, M.M., Cockcroft, S. and Gomperts, B.D. (1986) Nature 319, 504–507.
[7] Smith, C.D., Cox, C.C. and Snyderman, R. (1986) Science 232, 97–100.
[8] Strnad, C.F. and Wong, K. (1985) Can. J. Physiol. Pharmacol. 63, 1543–1546.
[9] Bradford, P.G. and Rubin, R.P. (1985) Mol. Pharmacol. 27, 74–78.
[10] Berridge, M.J. (1983) Biochem. J. 212, 849–858.
[11] Kishimoto, A., Kajikawa, N., Shiota, M. and Nishizuka, Y. (1983) J. Biol. Chem. 257, 13341–13348.
[12] Berridge, M.J., Downes, C.P. and Hanley, M.R. (1982) Biochem. J. 206, 587–595.
[13] Drummond, A.H., Bushfield, M. and MacPhee, C.H. (1984) Mol. Pharmacol. 25, 201–208.
[14] Michell, B. (1986) Nature 319, 176–177.
[15] Volpi, M., Naccache, P.H., Molski, T.F.P., Shefcyk, J., Huang, C.-K., Marsh, M.L., Munoz, J., Becker, E.L. and Sha'afi, R.I. (1985) Proc. Natl. Acad. Sci. USA 82, 2708–2712.
[16] Smith, C.D., Lane, B.C., Kusaka, I., Verghese, M.W. and Snyderman, R. (1985) J. Biol. Chem. 260, 5875–5878.
[17] Blackmore, P.F., Bocckino, S.B., Waynick, L.E. and Exton, J.H. (1985) J. Biol. Chem. 260, 14477–14483.
[18] Wong, K. (1983) Can. J. Biochem. Cell Biol. 61, 569–578.
[19] Berridge, M.J. (1984) Biochem. J. 220, 345–360.

[20] Smigel, M.D., Ferguson, K.M. and Gilman, A.G. (1985) in: Advances in Cyclic Nucleotide and Protein Phosphorylation Research (Cooper, D.M.F. and Seamon, K.B. eds) pp.103–111, Raven, New York.
[21] Howlett, A.C., Sternweis, P.C., Macik, B.A., Van Arsdale, P.M. and Gilman, A.G. (1979) J. Biol. Chem. 254, 2287–2295.
[22] Bokoch, G.M., Katada, T., Northup, J.K., Hewlett, E.L. and Gilman, A.G. (1983) J. Biol. Chem. 258, 2072–2075.
[23] Bigay, J., Deterre, P., Pfister, C. and Chabre, M. (1985) FEBS Lett. 191, 181–185.
[24] Sternweis, P.C. and Gilman, A.G. (1982) Proc. Natl. Acad. Sci. USA 79, 4888–4891.