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# Contribution of G Protein Activation to Fluoride Stimulation of Phosphoinositide Hydrolysis in Human Neuroblastoma Cells

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**Abstract:** To examine the possibility that NaF enhances phosphoinositide-specific phospholipase C (PIC) activity in neural tissues by a mechanism independent of a guanine nucleotide binding protein ($G_p$), we have evaluated the contribution of $G_p$ activation to NaF-stimulated phosphoinositide hydrolysis in human SK-N-SH neuroblastoma cells. Addition of NaF to intact cells resulted in an increase in the release of inositol phosphates (450% of control values; $EC_{50}$ of ~8 m*M*). Inclusion of U-73122, an aminosteroid inhibitor of guanine nucleotide-regulated PIC activity in these cells, resulted in a dose-dependent inhibition of NaF-stimulated inositol lipid hydrolysis ($IC_{50}$ of ~3.5 $\mu M$). When added to digitonin-permeabilized cells, NaF or guanosine-5′-*O*-thiotriphosphate (GTPγS) resulted in a three- and sevenfold enhancement, respectively, of inositol phosphate release. In the combined presence of optimal concentrations of NaF and GTPγS, inositol phosphate release was less than additive, indicative of a common site of action. Inclusion of 2–5 m*M* concentrations of guanosine-5′-*O*-(2-thiodiphosphate) (GDPβS) fully blocked phosphoinositide hydrolysis elicited by GTPγS, whereas that induced by NaF was partially inhibited (65%). However, preincubation of the cells with GDPβS resulted in a greater reduction in the ability of NaF to stimulate inositol phosphate release (87% inhibition). Both GTPγS and NaF-stimulated inositol phosphate release were inhibited by inclusion of 10 $\mu M$ U-73122 (54–71%). The presence of either NaF or GTPγS also resulted in a marked lowering of the $Ca^{2+}$ requirement for activation of PIC in permeabilized cells. These results indicate that in SK-N-SH cells, little evidence exists for direct stimulation of PIC by NaF and that the majority of inositol phosphate release that occurs in the presence of NaF can be attributed to activation of $G_p$. **Key Words:** Inositol phospholipid—Phosphoinositide-specific phospholipase C—Fluoride—Guanine nucleotide—Neuroblastoma—Aminosteroid U-73122. **Fisher S. K. et al.** Contribution of G protein activation to fluoride stimulation of phosphoinositide hydrolysis in human neuroblastoma cells. *J. Neurochem.* **60,** 1800–1805 (1993).

In the CNS, most cell-surface receptors that couple to the activation of phosphoinositide-specific phospholipase C (PIC) do so via an intervening G protein ($G_p$)[1] (for review, see Fisher et al., 1992). One agent used to study the involvement of $G_p$ in the absence of receptor activation is fluoride, which in the form of $AlF_4^-$ is presumed to mimic the γ-phosphate group of GTP, thereby promoting the dissociation and activation of the G protein (Bigay et al., 1987). $AlF_4^-$ has been demonstrated to enhance markedly the regulation of partially purified brain PIC by $G_q$ (Smrcka et al., 1991). The addition of NaF has previously been shown to activate phosphoinositide hydrolysis in several neural preparations (Hepler and Harden, 1986;

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*Abbreviations used:* GDPβS, guanosine-5′-*O*-(2-thiodiphosphate); $G_p$, guanine nucleotide binding protein(s) that regulates phosphoinositide-specific phospholipase C activity; GTPγS, guanosine-5′-*O*-(3-thiotriphosphate); $IP_1$, 1-D-*myo*-inositol monophosphate; $IP_2$, 1-D-*myo*-inositol bisphosphate; $IP_3$, 1-D-*myo*-inositol trisphosphate; $IP_4$, 1-D-*myo*-inositol tetrakisphosphate; KGEH buffer, potassium glutamate–EGTA–HEPES; PIC, phosphoinositide-specific phospholipase C; U-73122, 1-[6-[[17β-3-methoxyestra-1,3,5(10)-trien-17-yl]-amino]hexy]-1*H*-pyrrole-2,5-dione; U-73343, 1-[6-[[17β-3-methoxyestra-1,3,5(10)-trien-17-yl]-amino]hexyl]-2,5-pyrrolidinedione. For the inositol phosphates, isomeric positioning of phosphate groups is indicated when appropriate. The inositol phosphate nomenclature adopted is that of the Chilton Convention (see Agranoff et al., 1985).

[1] More than one G protein may regulate PIC activity. The identity of the G protein that regulates PIC activity in SK-N-SH cells is currently unknown.

Martin et al., 1986; Litosch, 1987; Godfrey and Watson, 1988; Gonzales and Crews, 1988; Jope, 1988; Claró et al., 1990). However, the extent of NaF-stimulated phosphoinositide hydrolysis that is directly attributable to activation of $G_p$ remains uncertain because the ability of guanosine-5′-*O*-(2-thiodiphosphate) (GDPβS) to block this stimulation was not evaluated in these studies. Recently, the concept that NaF stimulation of inositol lipid hydrolysis in neural tissues occurs through activation of $G_p$ has been challenged. Li et al. (1990) observed that whereas phosphoinositide hydrolysis stimulated by the addition of guanosine-5′-*O*-(3-thiotriphosphate) (GTPγS) to brain membranes could be inhibited by inclusion of GDPβS, that due to NaF addition was unaffected. In the same membrane preparation, it was demonstrated that NaF- and GTPγS-mediated stimulations of phosphoinositide hydrolysis were additive, a result inconsistent with a common site of action for these two agents. From these observations, the authors concluded that NaF-stimulated phosphoinositide hydrolysis in brain membranes occurs through a mechanism that is independent of $G_p$. Consistent with this possibility, Carter et al. (1990) have demonstrated that NaF can directly activate a partially purified preparation of brain PIC. Furthermore, evidence that NaF activation of PIC activity is secondary to the mobilization of intracellular $Ca^{2+}$ in PC12 cells has been presented (Bencherif and Lukas, 1991).

Because of the uncertainty regarding the mechanism by which NaF stimulates inositol lipid hydrolysis in neural tissues, in the present study we have evaluated the contribution that activation of $G_p$ makes to NaF-stimulated phosphoinositide hydrolysis in human SK-N-SH neuroblastoma using both biochemical and pharmacological approaches. SK-N-SH cells are ideally suited to address this issue because they exhibit a robust stimulation of phosphoinositide hydrolysis and, when permeabilized, can be used for studies of the guanine nucleotide regulation of PIC activity (Akil and Fisher, 1989; Fisher et al., 1989). In addition, we have used a novel aminosteroid, U-73122 {1-[6-[[17β-3-methoxyestra-1,3,5(10)-trien-17-yl]amino]hexyl]-1*H*-pyrrole-2,5-dione}, which has recently been demonstrated to inhibit preferentially GTPγS-stimulated PIC activity in these cells (Thompson et al., 1991). In contrast to previous reports that have indicated an anomalous mechanism for NaF activation of phosphoinositide hydrolysis in neural tissues, in the present study we report that activation of $G_p$ can account for at least 87% of NaF-stimulated inositol phosphate release in SK-N-SH cells.

## MATERIALS AND METHODS

### Materials

*myo*-[2-$^3$H]Inositol (15 Ci/mmol) was obtained from American Radiolabeled Chemicals (St. Louis, MO, U.S.A.). Digitonin and ATP were obtained from Sigma Chemical Co. (St. Louis). Oxotremorine-M was purchased from Research Biochemicals, Inc. (Natick, MA, U.S.A.). U-73122 and U-73343 {1-[6-[[17β-methoxyestra-1,3,5(10)-trien-17-yl]amino]hexyl]-2,5-pyrrolidinedione} were generously provided by Dr. J. E. Bleasdale of the Upjohn Co. (Kalamazoo, MI, U.S.A.). GTPγS and GDPβS were from Boehringer Mannheim (Indianapolis, IN, U.S.A.). Dowex AG1-X8 (100–200 mesh, formate form) was obtained from Bio-Rad (Rockville Center, NY, U.S.A.). Tissue culture supplies were purchased from Corning Glass Works (Corning, NY, U.S.A.). Powdered Dulbecco's modified Eagle's medium and fetal calf serum were obtained from GIBCO (Grand Island, NY, U.S.A.). Free fura-2 was obtained from Molecular Probes (Eugene, OR, U.S.A.).

### Cell culture conditions

Human SK-N-SH neuroblastoma cells (passage number unknown) were cultured under conditions that have been previously described (Fisher and Snider, 1987). Cells that were 10–20 days postpassage were used for all experiments. After aspiration of culture medium, cells were detached from the tissue culture flasks by addition of Puck's $D_1$ solution (Honegger and Richelson, 1976), collected by centrifugation (300 *g* for 1 min), and, unless stated otherwise, resuspended in buffer A (142 m*M* NaCl, 5.6 m*M* KCl, 2.2 m*M* $CaCl_2$, 3.6 m*M* $NaHCO_3$, 1 m*M* $MgCl_2$, 5.6 m*M* D-glucose, and 30 m*M* sodium HEPES buffer, pH 7.4).

### Measurement of phosphoinositide hydrolysis

SK-N-SH cells were prelabeled for 2 or 3 days at 37°C in Dulbecco's modified Eagle's medium with fetal calf serum containing 10 μCi/ml of [$^3$H]inositol in an atmosphere of 90% air/10% $CO_2$. Under these conditions, the labeling of the inositol lipids achieves isotopic equilibrium (Fisher et al., 1990). For intact cells, the accumulation of a total $^3$H-inositol phosphate fraction was monitored in the presence of $Li^+$, as previously described (Thompson and Fisher, 1990). Identification of the individual inositol phosphate isomers present in such fractions was as previously reported (Fisher et al., 1990). For measurement of inositol lipid hydrolysis in permeabilized cells, the prelabeled cells were washed once with Puck's $D_1$ solution and then resuspended in potassium glutamate–EGTA–HEPES (KGEH) buffer (139 m*M* potassium glutamate, 2 m*M* ATP, 4 m*M* $MgCl_2$, 10 m*M* LiCl, 10 m*M* EGTA, and 30 m*M* sodium HEPES buffer, pH 7.4) containing 20 μ*M* digitonin. Cells were permeabilized for 5 min at 37°C at a protein concentration of ~3–4 mg/ml. Permeabilized cells were then centrifuged and washed with an equal volume of KGEH buffer (minus digitonin) and then resuspended in the same buffer. Incubations were routinely allowed to proceed for 30 min, after which time reactions were terminated and inositol phosphate release was quantified as previously described (Fisher et al., 1989). The free $Ca^{2+}$ concentration (determined directly by the addition of fura-2) was routinely maintained at ~60 n*M* (10 m*M* EGTA:2.0 m*M* $CaCl_2$). In experiments in which the free $Ca^{2+}$ concentrations were varied, values of ~10, 30, 60, 100, 250, and 1,000 n*M* were obtained at $Ca^{2+}$/EGTA molar ratios of 0, 0.15, 0.20, 0.25, 0.40, and 0.55, respectively, with EGTA maintained at a concentration of 10 m*M*.

### Data analysis

Data are mean ± SEM values for the number of separate experiments performed. Dose–response curves were analyzed by the GraphPad InPlot curve-fitting program. Student's two-tailed *t* tests were used to evaluate the statistical differences of the means of paired sets of data.

Protein content was determined by the method of Geiger and Bessman (1972).

## RESULTS

### Intact cells

Addition of 20 m*M* NaF to SK-N-SH cells that had been labeled with [$^3$H]inositol to isotopic equilibrium resulted in a linear accumulation of inositol phosphates until 30 min of incubation, the interval routinely selected. Inclusion of NaF increased the release of inositol phosphates to 450 ± 60% of control values, whereas in the presence of 1 m*M* oxotremorine-M, the corresponding value was 1,570 ± 190% (n = 10). Half-maximal increases in inositol phosphate release were obtained at a NaF concentration of 8 ± 2 m*M* (n = 3; Fig. 1). HPLC analysis of inositol phosphate isomers formed indicated that the 1 and 3 isomers of 1-D-*myo*-[$^3$H]inositol monophosphate ([$^3$H]IP$_1$) (an enantiomeric pair), [$^3$H]I(4)P$_1$, and 1-D-myo-[$^3$H]-inositol bisphosphate ([$^3$H]IP$_2$) constituted 98% of the radioactivity recovered. Radiolabel in the 1,4,5 isomer of 1-D-*myo*-[$^3$H]inositol trisphosphate ([$^3$H]IP$_3$), [$^3$H]I(1,3,4)P$_3$, and 1-D-*myo*-[$^3$H]inositol tetrakisphosphate ([$^3$H]IP$_4$) made up the remainder. Similar proportions were obtained following addition of oxotremorine-M (see also Fisher et al., 1990).

Inclusion of U-73122 resulted in a dose-dependent inhibition of NaF-stimulated inositol phosphate release, with an IC$_{50}$ value of ~5 $\mu$*M* (Fig. 2). U-73122 inhibited both NaF- and oxotremorine-M-stimulated inositol phosphate release to a similar extent. Thus, in seven separate experiments, addition of 10 $\mu$*M* U-73122 resulted in a 60 ± 3 and 69 ± 7% inhibition of the NaF and oxotremorine-M responses, respectively. The succinimide derivative of U-73122, i.e., U-73343, had no significant inhibitory effect on NaF-stimulated inositol phosphate release when added at a concentration of 10 $\mu$*M* (6 ± 3%, n = 3). In contrast,

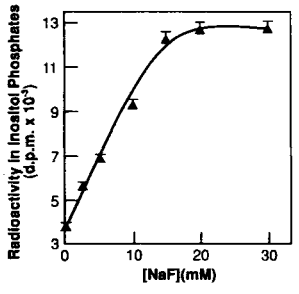


**FIG. 1.** Dose dependence of NaF stimulation of phosphoinositide hydrolysis. Intact cells were incubated for 30 min at 37°C in the presence of NaF at the concentrations indicated. Data are mean ± SEM (bars) values for triplicate replicates. Maximal activation of phosphoinositide hydrolysis was consistently obtained at 15–20 m*M* concentrations of NaF. At concentrations of NaF of >20 m*M*, an inhibition of inositol phosphate release was observed in two of four experiments.

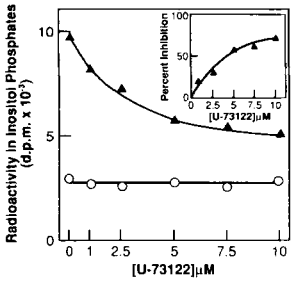


**FIG. 2.** Dose dependence of U-73122 inhibition of NaF-stimulated phosphoinositide hydrolysis. Intact cells (0.2 mg of protein/ml) were incubated with 20 m*M* NaF for 30 min in the absence or presence of U-73122 at the concentrations indicated. Data are mean values for triplicate replicates. Basal (○) or NaF-stimulated (▲) inositol phosphate release is shown. The release of inositol phosphates at zero-time was 2,218 dpm. **Inset:** Calculated percent inhibition of NaF-stimulated phosphoinositide hydrolysis as a function of U-73122 concentration.

an 18 ± 2% inhibition of oxotremorine-M-stimulated inositol phosphate release occurred in the presence of U-73343, which can be attributed to a direct effect of the aminosteroid on the muscarinic receptor itself (Thompson et al., 1991).

### Permeabilized cells

Addition of NaF to digitonin-permeabilized cells also elicited a release of inositol phosphate that was approximately linear for at least 30 min and was optimal at a concentration of 10 m*M* (EC$_{50}$ of ~4 m*M*; data not shown). The release of inositol phosphates obtained under these conditions was 417 ± 47% of control values, whereas that induced by addition of an optimal concentration of GTP$\gamma$S (20 or 200 $\mu$*M*) was 810 ± 64% (n = 9). The EC$_{50}$ value for GTP$\gamma$S stimulation of phosphoinositide hydrolysis was 2 $\mu$*M*. As for intact cells, the major inositol phosphate isomers formed were [$^3$H]I(1/3)P$_1$, [$^3$H]I(4)P$_1$, and [$^3$H]IP$_2$; relatively little formation of inositol polyphosphates was observed.

Both NaF- and GTP$\gamma$S-stimulated inositol phosphate releases were inhibited by inclusion of GDP$\beta$S (Fig. 3). Addition of GDP$\beta$S (2 or 5 m*M*) fully blocked GTP$\gamma$S-stimulated phosphoinositide hydrolysis, whereas only a partial inhibition (65%) of NaF-stimulated inositol phosphate release was observed. However, when cells were preincubated with GDP$\beta$S for 10 min (to displace residual GDP), NaF-stimulated inositol phosphate release was inhibited by 87 ± 3% (n = 3; see Fig. 3). The basal release of inositol phosphates was also inhibited by 30 ± 13% following inclusion of 5 m*M* GDP$\beta$S (n = 8).

If NaF and GTP$\gamma$S both act primarily to dissociate G$_p$, thereby causing its activation, it would be anticipated that inositol phosphate release obtained in the presence of optimal concentration of the two agents would be less than additive. In six separate experiments, inositol phosphate release obtained in the pres-

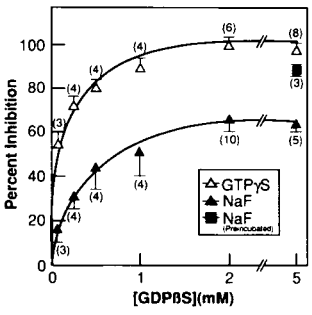


**FIG. 3.** GDP$\beta$S inhibits both NaF- and GTP$\gamma$S-stimulated inositol phosphate release. Digitonin-permeabilized SK-N-SH cells (0.75 mg of protein) were incubated in KGEH buffer [$Ca^{2+}$ = 60 n*M*] with either 10 m*M* NaF or 7 $\mu$*M* GTP$\gamma$S in the absence or presence of GDP$\beta$S at the concentrations indicated. Reactions were terminated after 30 min, and a total inositol phosphate fraction was isolated. Data are mean ± SEM (bars) values for the number of separate experiments indicated in parentheses.

ence of 10 m*M* NaF was 320 ± 40%, whereas the corresponding value for 20 $\mu$*M* GTP$\gamma$S was 626 ± 72%. A combination of these two agents resulted in an inositol phosphate release that was 651 ± 66% of control values (69 ± 2% of the theoretically additive values, $p < 0.001$).

The aminosteroid U-73122 has previously been demonstrated to inhibit guanine nucleotide-stimulated phosphoinositide hydrolysis in SK-N-SH cells, whereas direct activation of PIC by $Ca^{2+}$ addition is less affected (Thompson et al., 1991). Inclusion of the aminosteroid dose-dependently inhibited NaF-stimulated inositol phosphate release with an $IC_{50}$ value of ~2 $\mu$*M* (Fig. 4). NaF- and GTP$\gamma$S-stimulated phosphoinositide hydrolyses (n = 9) were inhibited by 54 ± 7 and 71 ± 3%, respectively, by inclusion of 10 $\mu$*M* U-73122. As observed following addition of GDP$\beta$S, inclusion of 10 $\mu$*M* U-73122 also inhibited the basal release of inositol phosphates (52 ± 4%, n = 9), indicating the presence of a guanine nucleotide component in this measure.

Because activation of $G_p$ is associated with a reduction in the $Ca^{2+}$ concentrations required for activation of PIC in SK-N-SH neuroblastoma cells (Fisher et al., 1989), we also determined the $Ca^{2+}$ concentration dependence of NaF-activated phosphoinositide hydrolysis (Fig. 5). Whereas the basal release of inositol phosphates was little influenced by $Ca^{2+}$ until its concentration exceeded 100–200 n*M*, concentrations of $Ca^{2+}$ found in the cytosol of quiescent cells (30–50 n*M*; see Fisher et al., 1989) were sufficient to support activation of PIC in the presence of either NaF or GTP$\gamma$S. Thus, both GTP$\gamma$S and NaF appear to lower the concentration of cytosolic $Ca^{2+}$ required for PIC activity.

## DISCUSSION

The results obtained in the present study strongly suggest that the stimulatory effect of NaF on phosphoinositide hydrolysis in SK-N-SH cells is mediated predominantly, if not exclusively, via the activation of $G_p$. Four lines of evidence support this conclusion. First, addition of 2 or 5 m*M* concentrations of GDP$\beta$S to permeabilized cells substantially inhibited (by 65%) NaF-stimulated inositol phosphate release and abolished that induced by GTP$\gamma$S. When cells were preincubated with GDP$\beta$S, a greater inhibition (87%) of NaF-enhanced phosphoinositide hydrolysis occurred. Second, when optimal concentrations of both NaF and GTP$\gamma$S were present in the incubation, the release of inositol phosphates was less than addi-

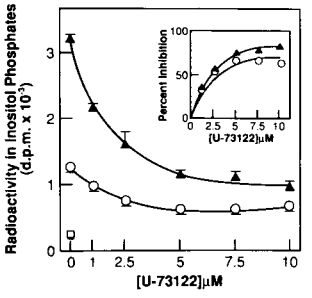


**FIG. 4.** Dose dependence of U-73122 inhibition of NaF-stimulated phosphoinositide hydrolysis. Digitonin-permeabilized SK-N-SH cells (0.15 mg of protein/ml) were incubated with either KGEH buffer (○) or with 10 m*M* NaF (▲) in the absence or presence of U-73122 at the concentrations indicated. Inositol phosphate release at zero-time is also shown (□). **Inset:** Calculated percent inhibition of basal and NaF-stimulated phosphoinositide hydrolysis as a function of U-73122 concentration.

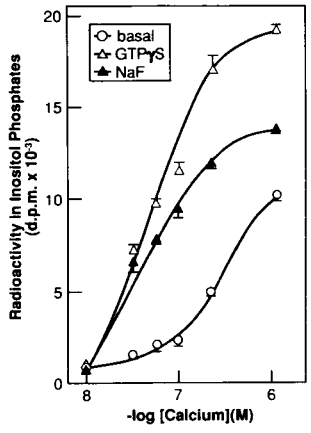


**FIG. 5.** $Ca^{2+}$ requirements for basal and NaF- and GTP$\gamma$S-stimulated PIC activity. Digitonin-permeabilized SK-N-SH cells (0.5 mg of protein) were incubated in KGEH buffer alone or in the presence of 10 m*M* NaF or 20 $\mu$*M* GTP$\gamma$S for 30 min at 37°C in the presence of $Ca^{2+}$ at the concentrations indicated (for details of the $Ca^{2+}$/EGTA buffers used, see Fisher et al., 1989). Data are mean ± SEM (bars) values for triplicate replicates from one of two experiments that gave similar results.

tive, suggesting a common site of action for these agents. Third, as previously observed for GTPγS in permeabilized SK-N-SH cells (Fisher et al., 1989), the addition of NaF resulted in a marked lowering of the $Ca^{2+}$ requirements necessary for PIC activation (Fig. 5). Guanine nucleotide activation has also been demonstrated to lower the $Ca^{2+}$ requirements for PIC in other nonneural tissue preparations (Bradford and Rubin, 1986; Uhing et al., 1986; Ali et al., 1989). Fourth, the ability of U-73122 to inhibit potently NaF-stimulated inositol phosphate release in both intact and permeabilized SK-N-SH cells also points to the ability of fluoride to activate directly $G_p$. U-73122 has previously been demonstrated to inhibit PIC-dependent processes in both neural and nonneural tissues at a postreceptor level (Bleasdale et al., 1990; Smith et al., 1990; Thompson et al., 1991). In both neutrophils and SK-N-SH cells, the ability of the aminosteroid to inhibit GTPγS- rather than $Ca^{2+}$-activated PIC activity indicates a preferential, although not absolute, site of action of the agent at the level of the $G_p$–PIC interaction (Bleasdale et al., 1990; Thompson et al., 1991). U-73122 inhibited NaF-stimulated inositol phosphate release to an extent similar to that observed for a muscarinic agonist in intact cells and for GTPγS in permeabilized cells. The close structural analogue, U-73343, which has little or no inhibitory effect on either agonist- or GTPγS-stimulated phosphoinositide hydrolysis (Thompson et al., 1991), was also without effect on NaF-stimulated inositol phosphate release. Taken collectively, these results suggest that the majority of inositol phosphate release that occurs in the presence of NaF can be attributed to activation of $G_p$.

From an analysis of inositol phosphate isomer formation, both NaF and muscarinic agonists appear to activate similar pathways of phosphoinositide breakdown in SK-N-SH cells. Addition of NaF resulted predominantly in the production of $I(1/3)P_1$, $I(4)P_1$, and $IP_2$ in both intact and digitonin-permeabilized cells, as previously observed for muscarinic agonists (Fisher et al., 1990). Although much less radioactivity was recovered in $I(1,4,5)P_3$, $I(1,3,4)P_3$, and $IP_4$, these isomers were consistently detected in the presence of NaF. The minimal contribution that polyphosphoinositide hydrolysis makes to inositol phosphate formation in the presence of NaF (60–62%) is similar to that calculated for muscarinic agonist-stimulated inositol lipid hydrolysis in these cells (68–70%; see also Fisher et al., 1990). Addition of NaF also resulted in an enhanced release of inositol phosphates under conditions in which intracellular concentrations of $Ca^{2+}$ were maintained by the use of $Ca^{2+}$/EGTA buffers (Fig. 5). Thus, unlike in PC12 cells (Bencherif and Lukas, 1991), a secondary mobilization of intracellular $Ca^{2+}$ in SK-N-SH cells cannot account for the ability of NaF to stimulate phosphoinositide hydrolysis.

The characteristics of NaF-stimulated inositol lipid hydrolysis observed for SK-N-SH cells differ considerably from those obtained for brain membranes (Li et al., 1990). For example, in the latter preparation stimulation of inositol phosphate release by NaF addition is insensitive to GDPβS and additive in the presence of GTPγS, whereas no additional $Ca^{2+}$ sensitivity of PIC is conferred in the presence of NaF. Moreover, in brain membranes, NaF activates inositol phosphate release to a greater extent than GTPγS (Gonzales and Crews, 1988; Li et al., 1990), whereas in SK-N-SH cells the order of effectiveness is reversed. Conceivably, some of these differences may be explained by the presence of different forms of PIC and $G_p$ and/or an alteration in the characteristics of $G_p$-regulated PIC activity following the isolation of brain membranes. In the latter context, two recent studies have indicated that $G_p$-regulation of PIC activity in reconstituted systems exhibits some marked differences from that observed in less perturbed systems such as permeabilized cells. For example, the regulation of PIC following $G_q$ addition does not result in any change in the $Ca^{2+}$ sensitivity of the enzyme, indicating the need for other membrane components that are removed during purification (Smrcka et al., 1991; Taylor et al., 1991). Furthermore, following purification, $G_q$ fails to bind significant amounts of GTPγS, an observation that accounts for the relative inability of this guanine nucleotide to enhance PIC activity when the latter is reconstituted with $G_q$ (Smrcka et al., 1991; Taylor et al., 1991). In contrast, $AlF_4^-$ is an effective promoter of phosphoinositide hydrolysis in this preparation. Thus, in both reconstitution assays and in brain membranes, fluoride more effectively promotes phosphoinositide hydrolysis than GTPγS. One possible explanation to reconcile these observations would be that a tightly bound pool of GDP, which is not readily displaced by GTPγS but is still accessible to fluoride, remains associated with $G_p$. Regardless of the mechanism involved, demonstration of an absolute requirement for the presence of $G_q$ in the stimulation of PIC by $AlF_4^-$ points to a direct involvement of the G protein (Smrcka et al., 1991; Waldo et al., 1991). Our results with SK-N-SH cells are also fully compatible with a central role for $G_p$ activation in NaF stimulation of phosphoinositide hydrolysis.

Previous work has indicated that NaF may have additional effects on the resynthesis of inositol lipids (Claró et al., 1990) and metabolism of inositol phosphates (Tiger et al., 1990). However, based on the results obtained in the present study, there appears little reason to challenge a central role for $G_p$ activation in the initiation of NaF-stimulated phosphoinositide hydrolysis in SK-N-SH cells.

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