Sodium Fluoride Mimics Effects of Both Agonists and Antagonists on Intact Human Platelets by Simultaneous Modulation of Phospholipase C and Adenylate Cyclase Activity

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Using intact human platelets, we studied the effect of sodium fluoride (NaF) on platelet aggregation and release reaction and correlated the functional changes to intracellular events specific for either agonist-induced or antagonist-induced platelet responses. At lower concentrations, with a peak activity between 30 and 40 mmol/L, NaF induced aggregation and release of adenosine 5′-triphosphate (ATP) that was associated with increased formation of inositol phosphates, a rise in cytosolic free Ca2+, and phosphorylation of 20-kd and 40-kd proteins. At NaF concentrations >40 mmol/L, aggregation and ATP release decreased dose-dependently in parallel with a decrease in Ca2+ mobilization, whereas neither inositol phosphate formation nor 40-kd protein phosphorylation was reduced. At these concentrations, NaF caused a transient rise in platelet cyclic adenosine 3′,5′-monophosphate (cAMP) levels that was sufficient to account for the observed reduction in Ca2+ mobilization, aggregation, and ATP release. Stimulated cAMP levels started declining rapidly within 30 seconds of addition of NaF, however. Similarly, prostacyclin (PGI2)-induced cAMP accumulation was temporarily enhanced but subsequently suppressed by NaF, suggesting either stimulation of a cAMP phosphodiesterase or delayed inhibition of adenylate cyclase. Evidence for the latter was provided by the finding that NaF pretreatment of platelets resulted in partial inhibition of PGI2-stimulated cAMP formation in the presence of the cAMP phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (MIX). We conclude that NaF exerts a dual (stimulatory and inhibitory) effect on adenylate cyclase in intact platelets that is accompanied by simultaneous activation of a phospholipase C-catalyzed hydrolysis of membrane inositol phospholipids, Ca2+ mobilization, activation of protein kinase C, and arachidonic acid liberation, whereas the other is related to the formation of cyclic adenosine 3′,5′ monophosphate (cAMP) catalyzed by adenylate cyclase.34 Adenylate cyclase activity is subject to regulation by two distinct guanine nucleotide-binding proteins (also referred to as G or N proteins) that couple the enzyme to specific membrane receptors and allow receptor expression as either N-mediated stimulation of N-mediated inhibition of its catalytic subunit.57 In addition, more recent evidence gave rise to the hypothesis that a guanine nucleotide-binding protein might also constitute the link that couples stimulatory platelet receptors to phospholipase C activation and/or Ca2+ mobilization.8-10 Fluoride activates N proteins by promoting the dissociation of their subunits.5,11-13 In isolated platelet membranes, sodium fluoride (NaF) both stimulates and inhibits adenylate cyclase through activation of N, and N, respectively.12,13 Conflicting results have been reported regarding the influence of NaF on intact platelets. Enhanced cAMP formation has been described14 as have NaF-induced mobilization of cytosolic free Ca2+, platelet aggregation, and serotonin release.15-17 These findings led us to investigate the effects of NaF on platelet function further, with particular attention to intracellular regulatory processes. In this article we demonstrate that NaF has a concentration-dependent, biphasic effect on platelet activation as a result of simultaneous modulation of phospholipase C and adenylate cyclase activity. We suggest that NaF-sensitive N proteins are not only involved in the regulation of adenylate cyclase but may also play a role in phospholipase C activation.

MATERIALS AND METHODS

Reagents. [2,8-3H]Adenine (17.7 Ci/mmol) and [8-14C] cAMP (45.1 mCi/mmol) were obtained from New England Nuclear (Boston). Carrier-free [32P]orthophosphate (45.1 mCi/mmol) was obtained from New England Nuclear (Boston). Carrier-free [32P]orthophosphate (45.1 mCi/mmol) was obtained from New England Nuclear (Boston). Myo-[23H]inositol (13.8 Ci/mmol), and quin 2 acetoxymethyl tetraester (Quin 2/AM) came from Amersham International (Amersham, England). Insta- Gel II was from Packard Instrument B.V. Chemical Operations (Groningen, The Netherlands). AG 50W-X4 cation exchange resin (200 to 400 mesh) was obtained from Bio-Rad Laboratories (Richmond, CA), and Dowex 1×8 anion exchange resin (200 to 400 mesh) was obtained from Fluka (Buchs, Switzerland). Sepharose 2B was from Pharmacia Fine Chemicals (Uppsala, Sweden). Apyrase, arachidonic acid and adenosine 5′-diphosphate came from Sigma Chemical (St Louis) and 3-isobutyl-1-methyl-xanthine (MIX) from Janssen Chimica (Beerse, Belgium). Platelet-activating factor (1-O-hexadecyl-2-ace- tyln-3glycerol-1-phosphorycholine) was from Boehringer (Mann-
Preparation of platelet-rich plasma. Blood was obtained by clean venipuncture of healthy volunteers who denied having taken any drugs during the previous 2 weeks. Anticoagulants used were either 0.1 vol of 106 mmol/L of trisodium citrate, 0.03 vol of 0.3 mol/L of EDTA, or 0.14 vol of ACD buffer (85 mmol/L of trisodium citrate, 71 mmol/L of citric acid, 111 mmol/L of dextrose, pH 4.5) as specified below. Platelet-rich plasma (PRP) was prepared by centrifugation at 150 g for 10 minutes at 20 °C.

Stimulation of platelets by NaF. For stimulation of platelets by NaF, 0.1 vol of prepared reaction mixtures containing NaF in distilled water in the appropriate amount to yield final concentrations of NaF between 10 and 100 mmol/L as indicated was added to 0.9 vol of platelet suspensions. To preclude any influence by Na+ or by changes in osmolarity, the reaction mixtures were made up with NaCl to give 1 mol/L solutions; 0.1 vol of 1 mol/L of NaCl in distilled water was added to controls. These principles were retained throughout all experimental procedures.

Platelet aggregation and ATP release. Aliquots of 0.45 mL of citrated PRP together with 0.05 mL of reconstituted luciferin/luciferase reagent (40 mg in 1 mL of sterile water) were placed into aggregometer cuvettes and preincubated while being stirred for 1 minute at 37 °C in a Lumi-Aggregometer Model 400 (Chrono-Log, Havertown, PA). Then 0.055 mL of NaF/NaCl-containing reaction mixtures was added. Aggregation and adenosine triphosphate (ATP) release were monitored over 10 minutes by parallel recording of light transmission and luminescence displayed on a dual-channel chart recorder. The luminescence signal was calibrated by addition of light transmission and luminescence displayed on a dual-channel counter.

Measurement of inositol phosphates. NaF-induced changes in inositol phosphate formation were measured as described, with minor modifications.16,19 PRP obtained from 100 mL of freshly drawn blood anticoagulated with EDTA was centrifuged at 800 g for 10 minutes at 20 °C. The platelet pellet was resuspended in 3 mL of a modified Tyrode’s solution-HEPES buffer (134 mmol/L of NaCl, 12 mmol/L of NaHCO3, 2.7 mmol/L of KCl, 12 mmol/L of NaHCO3, 2 mmol/L of MgCl2, 5.5 mmol/L of glucose pH 6.8) containing BSA (3.5 mg/mL) and apyrase (60 μg/mL). For labeling, gel-filtered platelets (3 × 109/mL) were incubated with 32P04 (0.8 mCi/mL) for 1 hour at 37 °C. Thereafter, platelets were again gel-filtered using Ca2+-restored and PO43- -restored Tyrode’s solution without albumin and apyrase for elution. The platelet count was adjusted to 7 × 109/mL. Samples of 0.9 mL of the final platelet suspension were incubated while being stirred with 0.1 mL of NaF/NaCl-containing reaction mixtures at 37 °C for varying times. The incubation was terminated by addition of 0.1 mL of 3 mol/L of HClO4. The precipitated proteins were isolated by centrifugation at 10,000 g for 5 minutes and separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) using the Laemmli buffer system.23 Gels were stained with Coomassie brilliant blue, and the specific areas corresponding to the 40-kd and 20-kd proteins were excised and counted for Cerenkov radiation in a liquid scintillation counter.

Measurement of cytosolic free Ca2+ concentrations. Platelets were isolated from citrated PRP by gel filtration on Sepharose 2B using an elution buffer containing 10 mmol/L of HEPES, 145 mmol/L of NaCl, 5 mmol/L of KCl, 0.5 mmol/L of NaHPO4, and 5 mmol/L of glucose pH 7.4. For loading with Quin 2, gel-filtered platelets (~1.5 × 1010/mL) were incubated with 10 μmol/L of Quin 2/AM for 1 hour at 37 °C. To remove extraneous dye, platelets were again gel-filtered with the same elution buffer, which contained no added Ca2+ or Mg2+ in order to prevent disturbance of the fluorescence measurements due to formation of insoluble CaF2 or MgF2. The final platelet count was adjusted to ~1 × 1010/mL. NaF-induced changes in cytosolic free Ca2+ concentrations ([Ca2+]c) were measured as changes in Quin 2 fluorescence intensity after addition of 0.1 vol of NaF/NaCl-containing reaction mixtures in a fluorescence spectrophotometer (Locarte, England). Measurements were made at 37 °C with an excitation wavelength of 339 nm and an emission wavelength of 492 nm. Under these conditions, no autofluorescence of NaF was observed. The fluorescence signal was calibrated in separate samples in the absence of NaF essentially as described.18,26 Changes in [Ca2+]c, were expressed in percentage of basal [Ca2+]c that was measured in resting platelets prior to addition of NaF.

Measurement of [3H]c-AMP. For labeling, PRP anticoagulated with EDTA was incubated with [3H]adenine (15 μCi/mL) for 30 minutes at 20 °C. Labeling was terminated by centrifugation at 800 g for 10 minutes at 20 °C and resuspension of the platelet pellet in an equal amount of citrated autologous plasma. Aliquots of 0.45 mL of the final platelet suspension were incubated with 0.05 mL of NaF/NaCl-containing reaction mixtures at 37 °C for varying times. In some experiments, 10 mmol/L of MIX or 0.05 mmol/L of PGI2 was added to the platelet suspension prior to or following the
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Stimulation by NaF as indicated. The incubation was stopped by addition of 0.05 mL of 3 mol/L of trichloroacetic acid containing \([^{14}C]\) cAMP (0.2 \(\mu\)Ci/mL) for assessment of recovery. After thorough mixing, the precipitated proteins were removed by centrifugation at 10,000 g for 5 minutes, and total \(^{3}H\)- and \(^{14}C\)-radioactivity in the supernatant was determined by liquid scintillation counting. cAMP was isolated from the trichloroacetic acid extract by ion-exchange chromatography on Dowex AG 50W-X4 followed by ZnSO\(_4\)/Ba(OH\(_2\)) precipitation of adenine nucleotides other than cAMP as described.27,28 The precipitate was spun down by centrifugation at 10,000 g for 5 minutes and \(^{3}H\)- and \(^{14}C\)-radioactivity in the final supernatant was measured. Results were corrected for spillover and loss factors and expressed as percentage of basal \(^{[\text{H}]\text{adenine incorporation into cAMP that was measured under control conditions (addition of 0.1 vol of 1 mol/L of NaCl). Basal \[^{[\text{H}]\text{adenine incorporation into cAMP ranged between 0.06% and 0.12\%.}}\)

Measurement of total lactate dehydrogenase (LDH). Total LDH activity was measured spectrophotometrically as described.29 LDH activity was determined in citrated platelet-poor plasma (PPP) either after addition of 0.1 vol of 1 mol/L of NaCl or NaF to PPP or after incubation of PRP with 0.1 vol of 1 mol/L of NaCl or NaF for 10 minutes at 37 \(^{\circ}\)C and subsequent removal of platelets by centrifugation at 10,000 g for 5 minutes. For estimation of the maximal increase in LDH activity caused by lysis of the platelets, digitonin (50 \(\mu\)mol/L) was added to separate samples of PRP immediately after addition of NaCl or NaF. These samples were further processed as detailed previously.

RESULTS

Effect of NaF on platelet aggregation and ATP release. NaF caused irreversible aggregation of platelets and secretion of platelet granule contents as measured by ATP release in a strictly dose-related manner (Fig 1). The peak aggregation response was observed at NaF concentrations of ~30 mmol/L. At higher concentrations, the onset of aggregation was more rapid; maximal increase in light transmission was reduced, however, and aggregation was almost entirely inhibited at NaF concentrations >70 mmol/L. Moreover, at these concentrations, platelets became refractory to subsequent stimulation by thrombin, collagen, adenosine 5'-diphosphate (ADP), arachidonic acid, platelet-activating factor (PAF), and calcium ionophore A23187 added in threshold concentrations 1 minute after addition of NaF (data not shown). Threshold concentrations of these agonists were determined in NaCl-treated control samples. Shape change, reflected by the initial decrease in light transmission, occurred at all concentrations tested. As illustrated in Fig 1B, platelet shape change was delayed and prolonged at low concentrations of NaF whereas it occurred almost instantaneously at high concentrations. Platelet aggregation induced by 30 mmol/L of NaF was not inhibited by apyrase, acetylsalicylic acid, or the PAF antagonist CV-3988,30 in concentrations sufficient to inhibit aggregation by threshold concentrations of ADP, arachidonic acid, and PAF, respectively. In addition, the aggregation response to 30 mmol/L of NaF was not blocked by preincubation of PRP with a combination of high concentrations of apyrase (5 mg/mL), acetylsalicylic acid (0.2 mmol/L), and CV-3988 (20 \(\mu\)mol/L) for 3 minutes (data not shown), indicating that the NaF-induced aggregation was independent of ADP release, arachidonic acid metabolism, or endogenously synthesized PAF. The concentration–response curve of NaF-induced ATP release essentially paralleled that of the aggregation response, with the exception that its peak was shifted to 40 mmol/L of NaF and that, at high concentrations of NaF that almost completely inhibited aggregation, there was still a marked release of ATP. ATP release occurred more rapidly with increasing concentrations of NaF.

Time-related and concentration-related effects of NaF on inositol phosphate formation. NaF-induced phosphodiesteric cleavage of platelet membrane phosphoinositides was monitored by measuring the formation of radioactive inositol phosphates in \([^{1}H]\)inositol-labeled platelets. As demonstrated in time-progress experiments (Fig 2), NaF at low (30 mmol/L) and high (80 mmol/L) concentrations caused a rise in inositol 1-phosphate (IP1) and inositol 1,4-biphosphate (IP2) levels. The increase in IP2 was detectable after 30 seconds at both concentrations tested and preceded the rise in IP1. The formation of both IP1 and IP2 appeared to be...
delayed at lower concentrations of NaF. This observation was confirmed by the findings of dose–response experiments in which the concentration-dependent differences in inositol phosphate formation were compensated by prolongation of the incubation time from 5 to 10 minutes (Fig 3). A small increase in inositol 1,4,5-trisphosphate (IP3) was measured within 30 seconds of addition of 80 mmol/L of NaF. No clear-cut changes in IP3 formation were detected when platelets were stimulated with lower concentrations of NaF, however, NaCl in a final concentration of 100 mmol/L did not affect inositol phosphate formation (data not shown).

**Effect of NaF on protein phosphorylation.** Preliminary time-course experiments (data not shown) revealed that the 40-kd protein was maximally phosphorylated within about 4 minutes of addition of 100 mmol/L of NaF. In dose–response experiments (Fig 4), 32P-labeled platelets were therefore incubated with increasing concentrations of NaF for either 1, 4, or 10 minutes. A minor rise in 32P content of the 40-kd protein after incubation for 1 minute was observed only at NaF concentrations of ≥40 mmol/L. At these concentrations, 40-kd protein phosphorylation was maximal after 4 minutes, yielding ~350% to 500% of control values, and a marked dephosphorylation was measured on further prolongation of the incubation time to 10 minutes. In contrast, at low concentrations of NaF, 32P incorporation into the 40-kd protein continued to rise when the incubation time was extended from 4 to 10 minutes, reaching a maximum of ~450% of control values. These data indicate that the rate rather than the maximal degree of 40-kd protein phosphorylation was concentration-dependent. Only minor changes in 32P incorporation into the 20-kd protein were observed (data not shown), which was in accordance with the relatively small rises in [Ca2+], induced by NaF (see below); 20-kd
values. In contrast, at concentrations >40 mmol/L, there was a marked depression of NaF-induced Ca²⁺ mobilization that coincided with a dose-dependent exponential increase in peak cAMP levels. Because [³H]cAMP formation was determined as detailed in the Materials and Methods section. Basal [³H]adenine incorporation into cAMP was measured after incubation of platelets with 0.1 vol of 1 mol/L of NaCl for 20 seconds and was set equal to 100%. Values are given as means ± SE of triplicate incubations. The data shown are representative of at least three experiments.

**Time-related and concentration-related effects of NaF on cytosolic free Ca²⁺ and platelet cAMP levels.** As illustrated in Fig 5, NaF promoted a dose-dependent transient rise in platelet cAMP levels. Net cAMP formation peaked already within 10 to 20 seconds of addition of 100 mmol/L of NaF, whereas the much smaller maximal increment in platelets when stimulated with 30 mmol/L of NaF was observed after 30 seconds. Thus, it is likely that the results of dose–response experiments in which platelets were incubated with different concentrations of NaF for 20 seconds underestimate to a minor extent the effect of low concentrations of NaF on platelet cAMP levels.

The increase in [Ca²⁺], observed when platelets were stimulated with NaF was preceded by a lag period which in its length was inversely related to the concentration of NaF used. For evaluation of concentration-related effects of NaF on [Ca²⁺], peak [Ca²⁺], was calculated from the maximal Quin 2 fluorescence signal observed during 10 minutes following the addition of NaF.

Figure 6 gives an integrated view on the dose-related effects of NaF on peak [Ca²⁺], and peak cAMP levels. At low concentrations of NaF (<40 mmol/L), net cAMP formation was only little affected, whereas [Ca²⁺], increased in a dose-dependent manner to maximally 200% to 250% of basal values. In contrast, at concentrations >40 mmol/L, there was a marked depression of NaF-induced Ca²⁺ mobilization that coincided with a dose-dependent exponential increase in peak cAMP levels. Because [³H]cAMP formation was determined in PRP and [Ca²⁺], was measured in platelets suspended in low [Ca²⁺]-containing buffer using the fluorescent Ca²⁺ probe Quin 2. Results reflect maximal [Ca²⁺] measured during an observation period of 10 minutes after addition of various concentrations of NaF and are expressed in percentage of basal [Ca²⁺]. Points represent means ± SE of at least three experiments.

**NaF-induced inhibition of adenylate cyclase.** The preceding data show that NaF-treatment of platelets results in a transient rise in cAMP that is followed by a rapid decline to slightly elevated or basal levels (Fig 5). A similar time course was observed in the presence of PGI₂ (Fig 7). Incubation of platelets with 0.05 μmol/L of PGI₂ resulted in a rapid rise in cAMP levels that reached a maximum after 3 minutes and only gradually decreased during the following 10 minutes. Addition of 30 or 100 mmol/L of NaF to platelets that were preincubated with 0.05 μmol/L of PGI₂ for 3 minutes promoted a marked further dose-dependent increase in platelet cAMP levels that peaked after 10 seconds. At both NaF concentrations tested, however, the temporary enhancement of PGI₂-induced cAMP formation was followed by a rapid decrease in cAMP to clearly below the levels maintained by stimulation with PGI₂ alone. These data were suggestive of either an NaF-induced activation of a cAMP phosphodiesterase or a delayed inhibitory effect of NaF on adenylate cyclase activity. When platelets were preincubated with 10 mmol/L of MIX, a potent cAMP phosphodiesterase inhibitor,²⁰ for 4 minutes prior to exposure to either 100 mmol/L of NaCl or 30 mmol/L of NaF and 70 mmol/L of NaCl for an
Fig 7. Effect of NaF on prostacyclin (PGI2)-stimulated cAMP accumulation. [3H]Adenine-labeled platelets were incubated with 0.05 μmol/L of PGI2 for 3 minutes prior to addition of either 100 mmol/L of NaCl (□), 30 mmol/L of NaF + 70 mmol/L of NaCl (○), or 100 mmol/L of NaF (●). At the time points indicated, [3H]cAMP was determined as described in the Materials and Methods section. Results are expressed in percentage of basal [3H]adenine incorporation into cAMP and are given as means ± SE of triplicate incubations. The data shown are representative of three experiments.

Additional 4.5 minutes, a slow progressive rise in platelet cAMP levels attributable to cAMP phosphodiesterase inhibition was observed (Fig 8). The slightly larger rise measured on addition of NaF was probably due to minor stimulation of adenylate cyclase. When finally 0.05 μmol/L of PGI2 was added, cAMP levels progressively increased over the next 10 minutes in the NaCl-treated controls. In the NaF-treated samples, the further increase in cAMP achieved by addition of PGI2 was strikingly smaller, indicating that NaF indeed exerted a delayed inhibitory effect on adenylate cyclase. Our data, however, do not rule out that NaF, in addition, stimulates a low-affinity cAMP phosphodiesterase.

LDH measurements. Incubation of platelets with either 100 mmol/L of NaCl or 100 mmol/L of NaF did not cause a detectable rise in total LDH activity in citrated plasma, indicating that neither treatment caused measurable lysis of platelets.

DISCUSSION

We showed that NaF at optimal concentrations causes irreversible aggregation of platelets and secretion of granule contents as measured by ATP-release. The aggregation induced by NaF cannot be blocked by a combination of high concentrations of specific inhibitors that entirely abolishes aggregation by arachidonic acid, ADP, and PAF, respectively. Thus, it can be assumed that NaF exerts its agonistic effects on platelet function through a mechanism that is independent of the formation of cyclooxygenase products or the release of ADP or PAF.

An early event in agonist-induced platelet activation is the phospholipase C-catalyzed hydrolysis of phosphatidylinositol and its phosphorylated derivatives.3,4,32 This results in the generation of intermediate breakdown products that function as intracellular messenger molecules, among them 1P3, which mobilizes Ca2+ from intracellular storage sites,33,34 and diacylglycerol, the endogenous activator of protein kinase C.35,36 Stimulation of platelets by NaF increases the formation of inositol phosphates, indicating the activation of a phosphoinositide-specific phospholipase C. NaF-induced phosphoinositide turnover leads to activation of protein kinase C, as demonstrated by phosphorylation of its major substrate in platelets, a 40-kd protein,4,37,38 and a rise in cytosolic free Ca2+, which in turn triggers the phosphorylation of a 20-kd protein previously identified as a myosin light chain.39 In this study, we failed to detect any clear-cut changes in IP3 formation at NaF concentrations that evoked maximal Ca2+ mobilization. Because the rise in IP3 even on
stimulation of platelets by thrombin is only transient and minor as compared with the increase in IP2 and IP1,19 however, it is possible that the assay used is not sensitive enough to detect a slow rise in IP3 which, in addition, could be outbalanced by its rapid degradation. Therefore, the most likely explanation for the agonistic effects of NaF on platelet function would still be that NaF through activation of phospholipase C initiates an intracellular signal cascade that finally leads to platelet aggregation and release of granule contents. On the other hand, our data do not entirely exclude the existence of a Ca2+-mobilizing agent distinct from IP3 that is formed or activated on NaF stimulation of platelets.

The time lag that precedes the NaF effects related to platelet activation decreases with increasing concentrations of NaF and may thus reflect the time required for NaF to reach its site of action either within the platelet membrane or possibly within the platelet cytosol. Our data herein are consistent with previous studies reporting a delayed mobilization of cytosolic free Ca2+ in platelets,15 neutrophils,40 and hepatocytes41 exposed to NaF.

When the concentration of NaF is raised to >40 mmol/L, a dose-dependent reduction in the maximal amplitude of platelet aggregation is observed, and pretreatment of platelets with 70 to 100 mmol/L of NaF even prevents aggregation in response to stimulation by various other agonists. At these concentrations, NaF induces a dose-dependent transient rise in platelet cAMP, which probably accounts for the concomitant reduction in Ca2+ mobilization.3144 Inositol phosphate formation and 40-kd protein phosphorylation are not affected by the cAMP transients, suggesting that neither phospholipase C nor protein kinase C action is inhibited by a short-lived increase in cAMP levels. We thus conclude that the observed inhibition of platelet function at high concentrations of NaF is due to the increase in cAMP, which counteracts the mobilization of cytosolic free Ca2+ and mainly thereby inhibits aggregation and release reaction. Alternatively, NaF at high concentrations might interfere with the formation or activation of a Ca2+-mobilizing messenger distinct from IP3 or directly stimulate a Ca2+ pumping activity, thus reducing the cytosolic free Ca2+ concentration.

Platelet shape change is not and ATP release is only partially inhibited even at the highest concentrations of NaF tested, which would confirm the earlier observations that shape change and release reaction can occur with virtually no detectable rise in cytosolic free Ca2+ as measured by the fluorescent Ca2+ indicator Quin 2.2645

The rapid reversal of the NaF-stimulated increase in platelet cAMP levels raises the hypothesis that NaF may, in addition, either activate a cAMP phosphodiesterase or exert a delayed inhibitory effect on adenylyl cyclase. The latter possibility is confirmed by the observation that NaF pre-treatment of platelets partially inhibits PGI2-induced cAMP accumulation in the presence of MIX; this experiment, however, does not preclude an additional effect of NaF on a cAMP phosphodiesterase.

Because in platelet membrane preparations NaF has been shown to activate the stimulatory (N) as well as the inhibitory (N) guanine nucleotide-binding protein involved in the regulation of adenylyl cyclase activity,1213 it is possible that the dual effect of NaF on adenylyl cyclase in intact platelets mirrors the concentration-dependent, concomitant, or successive activation of both N and N. In extension of this hypothesis, we further suggest that a NaF-sensitive N protein may also play a role in the activation of phospholipase C.

We observed activation of phospholipase C by NaF independent of a rise in cytosolic free Ca2+ and despite high cAMP transients. In granulocyte membranes, an N protein is required for the stimulation of phosphoinositide-specific phospholipase C at resting cytosolic free Ca2+ concentrations.46

In conclusion, we demonstrated that NaF, as a result of simultaneous modulation of phospholipase C and adenylyl cyclase activity, has a biphasic, concentration-dependent effect on the function of intact human platelets. The action of NaF may be linked to its known capacity to activate N proteins.

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