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Platelet-derived growth factor stimulates Na^+/H^+ exchange and induces cytoplasmic alkalinization in NR6 cells

(pH$_i$ regulation/mitogen/amiloride/4',5'-dimethylfluorescein)

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ABSTRACT **Stimulation of Na^+/H^+ exchange by growth factors has been implicated as a mechanism allowing quiescent cells to resume growth because of a predicted elevation of intracellular pH (pH$_i$). We tested this prediction in NR6 cells by using a further development of our technique for pH$_i$ measurement, based on introduction of the fluorescent pH indicator 4',5'-dimethylfluorescein (pK_a = 6.75) coupled to dextran into the cytoplasm. Addition of the potent mitogens platelet-derived growth factor (PDGF) or serum to NR6 cells stimulates an amiloride-sensitive $^{22}Na^+$ uptake and causes an elevation of pH$_i$. The PDGF-dependent pH$_i$ increase follows a lag period of ≈2 min, reaches a maximal level within 10 min (ΔpH$_i$ ≈ 0.1 at an external pH of 7.18), and remains at this level for at least 1 hr. Serum addition initially produces a large elevation of pH$_i$, which later declines to a level similar to that obtained with PDGF. The effects of PDGF and serum are partially additive (ΔpH$_i$ ≈ 0.14). The magnitude of pH$_i$ elevation by PDGF decreases with increasing extracellular pH. Serum- and PDGF-dependent elevations of pH$_i$ are inhibited by amiloride and by eliminating Na^+ from the medium. Under conditions in which Na^+/H^+ exchange is inhibited, PDGF and serum induce an initial cytoplasmic acidification that does not show a lag period. The results show that a single purified growth factor, as well as serum, can promote a sustained elevation of pH$_i$ by stimulating Na^+/H^+ exchange. The extent of pH$_i$ elevation may be modulated by the concomitant stimulation by the growth factor of a process generating H^+ within the cell.**

Addition of growth factors to quiescent mammalian cells triggers emergence of the cells from the G_0/G_1 phase of the cell cycle and subsequent progression along a defined pathway culminating in DNA synthesis and mitosis. Recently, particular interest has focused on the hypothesis that elevation of intracellular pH (pH$_i$) may act as a mediator of growth factor action. Both serum and pure growth factors have been found to rapidly stimulate an electroneutral and amiloride-sensitive Na^+ uptake, suggesting a stimulation of Na^+/H^+ exchange (1–4). Since under physiological conditions there is a large Na^+ concentration gradient directed inward, stimulation of Na^+/H^+ exchange may lead to cytoplasmic alkalinization. This has been shown to occur in the sea urchin egg (5), in which an increase in pH$_i$ brought about by stimulation of Na^+/H^+ exchange on fertilization appears to provide a crucial stimulus for egg development.

Assessing cytoplasmic pH in cells that depend on growth factors for proliferation has been hampered by lack of suitable methods for the determination of pH$_i$ in cells grown in monolayer cultures. Schuldiner and Rozengurt (6) recently used the distribution of weak acids across the cell membrane to demonstrate cytoplasmic alkalinization by ≈0.15 pH unit on addition of a combination of mitogens to Swiss 3T3 cells. However, the weak acid distribution method has disadvantages because of limited temporal resolution and compartmentalization of the probe in subcellular organelles, with an additional technical complication for measurements in monolayer cultures because of the high ratio of external to internal volume. We have recently introduced a method suitable for continuous monitoring of pH$_i$ based on introduction into the cell cytoplasm of fluorescein-labeled dextran, which then serves as a sensitive fluorescent pH indicator (7). This method has now been improved by the preparation of the fluorescent pH indicator 4',5'-dimethylfluorescein-dextran (DMFD), the pK_a of which is closer to the pH$_i$ than that of fluorescein. We use the mouse 3T3 cell variant NR6 (8), which shows a potent mitogenic response to platelet-derived growth factor (PDGF) to demonstrate and characterize a PDGF-dependent alkalinization of the cytoplasm. NR6 cells are more resistant than 3T3 cells to the experimental manipulations required to introduce the fluorescent reporter molecule into the cells.

METHODS

Pure PDGF was prepared as described (9). The sources of other reagents can be found in ref. 7. NR6 cells were grown in Dulbecco's modified minimal essential medium (DME medium; GIBCO)/7% newborn calf serum/3% fetal calf serum containing penicillin at 100 units/ml and streptomycin at 100 μg/ml in 10% CO_2/90% air. During experiments, the cells were incubated in physiological salt solution [solution A; 130 mM NaCl/5.4 mM KCl/1 mM P_i/0.8 mM $MgSO_4$/1.8 mM $CaCl_2$/25 mM glucose/human serum albumin (0.5 mg/ml; Sigma 9511)/25 mM Hepes, pH 7.18].

Loading of Cells with DMFD. Cells (1–2 × 10^5 in 2 ml of medium) were seeded in bacteriological 35-mm dishes, each containing two small glass slides (0.1 × 0.9 × 2.5 cm), and were grown to confluency. The use of bacteriological dishes considerably increases the proportion of cells that adhere to the glass slide. The cells were not fed for 2–3 days before loading. Loading was carried out by osmotic lysis of pinocytic vesicles (10) as described by Rothenberg *et al.* (7) except that DMFD (20% solution) was used in place of fluorescein-dextran. After loading, the glass slides were returned to their original dishes (containing DME medium/serum that had been deprived of growth factors during the previous incubation with the cells) and incubated for at least 15 hr to allow recovery from the osmotic stress. Prior to experiments, the cells were incubated for 4–6 hr in the serum-free solution A to inactivate serum-dependent Na^+/H^+ exchange activity (11).

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Abbreviations: PDGF, platelet-derived growth factor; EGF, epidermal growth factor; DMFD, 4',5'-dimethylfluorescein isothiocyanate coupled to dextran; pH$_i$, intracellular pH; pH$_o$, extracellular pH.

Fluorometric Measurements. The glass slides were mounted in 3-ml cuvettes at an angle of 41° relative to the excitation beam, using a holder similar to that described by Ohkuma and Poole (12). Solutions were exchanged by perfusion of 3–6 cuvette volumes. When growth factors were tested, they were added at a concentration of 50 ng/ml for PDGF and of 6% (vol/vol) for fetal calf serum. Measurements were initiated about 30 min after insertion of the slide into the cuvette and were carried out at 37°C at λ_{ex} = 511 nm (band pass, 1 nm), λ_{em} = 545 nm (band pass, 20 nm), and signal damping of 10 sec in an Aminco SPF 500 ratio fluorometer. Data are corrected for light scattering, determined after quenching the fluorescence with 0.1 M HCl. The results are calculated based on an initial pH_i of 6.90 at an extracellular pH (pH_o) of 7.18 (see Fig. 4).

$^{22}Na^+$ Uptake Measurement. Confluent cells in 35-mm dishes were incubated for 4 hr in solution A. Uptake was then initiated by addition of 0.8 ml of solution A containing 5 mM ouabain and $^{22}Na^+$ (6–7 × 10^6 cpm/ml). Uptake was terminated and radioactivity was determined as described (7). Results represent means of triplicate determinations.

RESULTS

Mitogenic Response of NR6 Cells. NR6 cells are a Swiss mouse 3T3 cell variant that lacks epidermal growth factor (EGF) receptors but retains responsiveness to other growth-promoting agents (8). These cells also have other properties different from those of the parent 3T3 cell line. Most relevant among these properties, NR6 cells readily grow on the glass slides used for pH measurements and also survive well after loading with DMFD while 3T3 cells tend to aggregate and come off the glass slide during the loading procedure. Addition of PDGF to confluent growth factor-deprived NR6 cells strongly stimulates mitogenic response as determined by [^{3}H]thymidine incorporation. Half-maximal mitogenic effect of PDGF occurs at about 7 ng/ml and maximal stimulation with PDGF is about 70% of the stimulation obtained with 10% fetal calf serum (data not shown).

PDGF and Serum-Dependent $^{22}Na^+$ Uptake. Both serum and PDGF increase $^{22}Na^+$ uptake into NR6 cells (Fig. 1). The PDGF-stimulated uptake follows a lag period of about 2 min. Nearly maximal stimulation of $^{22}Na^+$ uptake occurs at 50 ng/ml, the concentration of PDGF used in this experiment. Half-maximal stimulation required PDGF at 10 ng/ml (data not shown), thus resembling the dose response for PDGF-dependent [^{3}H]thymidine incorporation. After the lag period, PDGF-stimulated $^{22}Na^+$ uptake is linear for about 5 min and then diminishes. Serum-dependent uptake also follows a short lag period. The initial rate of $^{22}Na^+$ uptake in the presence of serum is faster than that obtained with PDGF but the serum-dependent $^{22}Na^+$ uptake declines more rapidly than the PDGF-dependent uptake. Note that the experiment shown in Fig. 1 was carried out in the presence of ouabain to inhibit $^{22}Na^+$ extrusion by the Na^+/K^+ ATPase; under these conditions, linear kinetics of $^{22}Na^+$ uptake can proceed for only a limited period because of the increase with time in intracellular Na^+ concentration.

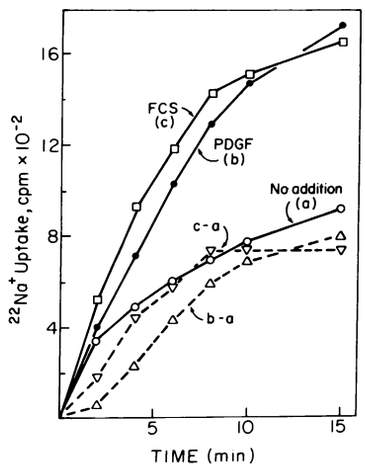

FIG. 1. Effect of PDGF (50 ng/ml) and dialyzed fetal calf serum (FCS; 6%) on $^{22}Na^+$ uptake by NR6 cells. Measurements were carried out in the presence of ouabain.

As shown in Fig. 2, addition of the Na^+/H^+ exchange inhibitor amiloride at 3 mM causes inhibition of the *net* serum- and PDGF-stimulated $^{22}Na^+$ uptake by about 40% but does not significantly affect the uptake in the absence of growth factors. Thus, the serum- and PDGF-dependent uptake is apparently mediated in part through a Na^+/H^+ exchange mechanism. Addition of both PDGF and serum results in partially additive stimulation of $^{22}Na^+$ uptake. When cells previously incubated with serum or PDGF for 40 min in the absence of ouabain are used, there is some decrease in both the total and the amiloride-sensitive growth factor-dependent $^{22}Na^+$ uptake, subsequently assayed for 8 min in the presence of ouabain and $^{22}Na^+$ (Fig. 2). Serum-dependent $^{22}Na^+$ uptake is decreased by about 70% while PDGF-dependent $^{22}Na^+$ uptake is reduced about 30%.

Measurements of Intracellular pH. We prepared the fluorescent pH indicator 4',5'-dimethylfluorescein isothiocyanate, which was then covalently coupled to hydrazine-derivatized dextran (M_r, 5,000). The synthesis and spectral characteristics of DMFD will be described elsewhere. The introduction of methyl groups into the fluorescein molecule increases the pK_a of the dye from 6.40 to 6.75, thus making it more sensitive to small changes in pH_i within the physiological range (13). The DMFD is introduced into the NR6 cell cytoplasm by pinocytosis of hypertonic medium followed by osmotic rupture of the pinosomes by using the method of Okada and Rechsteiner (10). After a recovery period of 15 hr or more, the cells are morphologically intact and the fluorescent dye appears diffusely distributed in the cell cytoplasm. The coupling of the dye to dextran prevents any leakage of the dye from the cells during the experimental period.

To calibrate the pH_i measurements in NR6 cells we used the method of Thomas *et al.* (14). Cells are treated with nigericin, an ionophore catalyzing K^+/H^+ exchange. At K_o^+ concentration (130 mM), which approximates intracellular K^+ concentration, the ionophore promotes equilibration of pH_i with the extra-

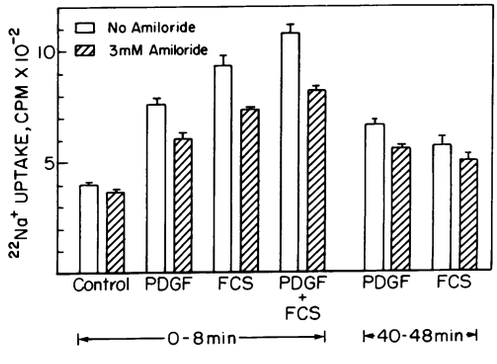

FIG. 2. Effect of amiloride (3 mM) on $^{22}Na^+$ uptake. Uptake was assayed for 6 min in the presence of ouabain; PDGF = 50 ng/ml, fetal calf serum (FCS) = 6%. FCS was freshly dialyzed against solution A. Data are mean ± SD of triplicate determinations.

cellular pH (pH_o). Gramicidin was also added to ensure equilibration of Na^+ and K^+ across the membrane. Indeed, the dependency on pH_o of the fluorescence emitted by the nigericin-treated cells (Fig. 3, pK_a = 6.70) resembles that of the free fluorophore (13). The fluorescence intensity prior to nigericin addition at pH_o = 7.18 (the pH_o normally used in all experiments) corresponds to a pH_i of 6.90 ± 0.05 (mean ± SD, n = 6). Also shown in Fig. 3 is the effect of changes in pH_o on pH_i in the absence of ionophores; it can be seen that under these conditions pH_i only partially tracks pH_o. A potential problem with the nigericin technique is that the ionophore will also alter the pH inside the lysosomes (12). Thus, if some of the DMFD is present in lysosomes after loading at 37°C, the nigericin technique will give an underestimate of cytoplasmic pH. Because pinocytic vesicles cannot fuse with lysosomes at reduced temperatures (15), we loaded cells with DMFD at 15°C for 15–30 min instead of the usual loading at 37°C for 10 min. The pH_i determined by the nigericin technique in cells that had been loaded at the two temperatures did not vary significantly, suggesting that in NR6 cells DMFD is not taken up into lysosomes in a significant amount even at 37°C.

Effect of PDGF and Serum on pH_i. DMFD-loaded cells show the same [^{3}H]thymidine incorporation as control cells after mitogenic stimulation with either serum or PDGF. Between 20 and 30 hr after loading, there is some increase in [^{3}H]thymidine incorporation in the absence of mitogens. This apparent mitogenic effect of the loading procedure diminishes after 42–48 hr. The PDGF and serum effects on pH_i to be described below did not vary significantly in cells that had been loaded 1 or 2 days prior to pH_i measurement, and the experiments were therefore carried out either 1 or 2 days after loading.

Addition of pure PDGF to DMFD-loaded NR6 cells causes an increase in pH_i following a characteristic lag period of about 2 min (Fig. 4). The duration of the lag period is almost unchanged at PDGF concentrations between 10 and 200 ng/ml (data not shown), indicating that it does not merely reflect the time required for the association of PDGF with its plasma membrane receptor. During this lag period, a slight initial

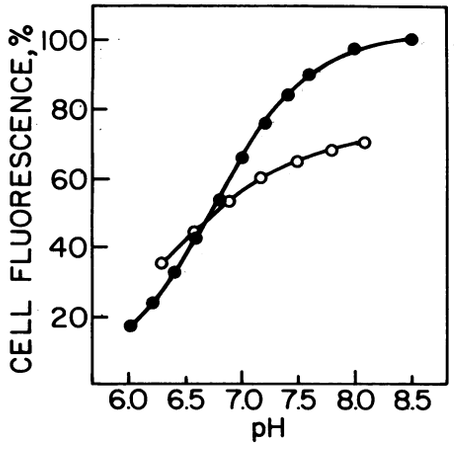

FIG. 3. Calibration of pH_i measurement in NR6 cells. ●, Cells containing DMFD were treated with nigericin at 10 μg/ml and gramicidin at 5 μg/ml in 130 mM KCl/10 mM NaCl/1 mM $MgSO_4$/1.8 mM $CaCl_2$/20 mM Hepes/10 mM 2-(*N*-morpholino)ethanesulfonic acid (Mes), pH 7.0. Subsequently, the pH_i was stepwise decreased to 6.0 and then stepwise increased to 8.5. ○, Cells were incubated in solution A/10 mM Mes at the indicated pH in the absence of ionophores. pH_i was then determined in these cells by determining the fluorescence intensity as a fraction of the maximum fluorescence intensity determined in the same cells at the end of the experiment in the presence of ionophores. Data are steady-state fluorescence at each pH.

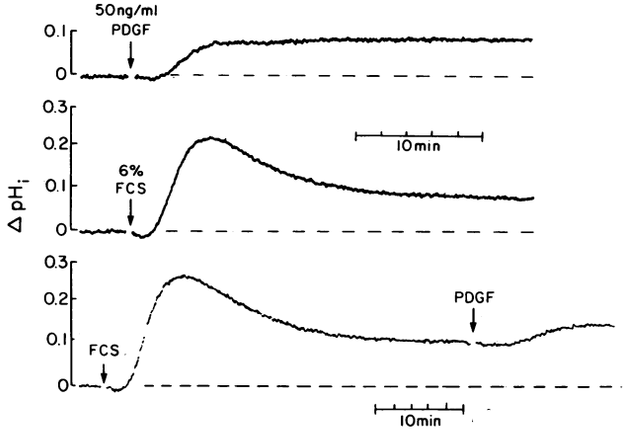

FIG. 4. Effect of PDGF and dialyzed fetal calf serum (FCS) on pH_i. The ΔpH_i scale represents the increase in pH_i from the resting value, estimated at 6.90.

acidification was observed in many experiments. A similar lag period was found for PDGF-dependent $^{22}Na^+$ uptake (Fig. 1). The subsequent elevation of pH_i is complete within 10 min, reaches approximately 0.1 pH unit, and remains at this level for at least 1 hr. The pH_i elevation elicited by PDGF ranged between 0.08 and 0.10 pH unit in different experiments. Epidermal growth factor (EGF; 300 ng/ml) and bovine insulin (1 μg/ml) have no effect on pH_i in NR6 cells under these conditions (data not shown). Addition of fetal calf serum produces a large initial elevation of pH_i, which then declines and stabilizes at about 0.10 unit above the initial pH. While the pattern shown in Fig. 4 for fetal calf serum was always observed, the initial pH_i elevation varied significantly (0.16–0.26 unit) in different experiments. The serum effect probably reflects the presence in serum of components other than PDGF because the effects of PDGF and serum on pH_i are partially additive (Fig. 4). PDGF and serum also give partially additive stimulation of $^{22}Na^+$ uptake (Fig. 2). As shown in Fig. 5, the elevation of pH_i by PDGF is half-maximal at about 10 ng/ml. A similar PDGF concentration causes half-maximal stimulation of both [^{3}H]thymidine incorporation and $^{22}Na^+$ uptake.

Fig. 6 shows the effect of the extracellular pH (pH_o) on pH_i in the presence and absence of PDGF. It can be seen that the increase in pH_i that is induced by PDGF progressively decreases at increasing pH_o from a ΔpH_i of 0.17 at pH_o = 6.8 to a ΔpH_i of about 0.03 at pH_o = 7.6. Note that PDGF addition not only elevates pH_i but also results in greater stability of pH_i to changes in pH_o.

Amiloride Sensitivity of PDGF and Serum-Dependent Cytoplasmic Alkalinization. Measuring the effect of amiloride on

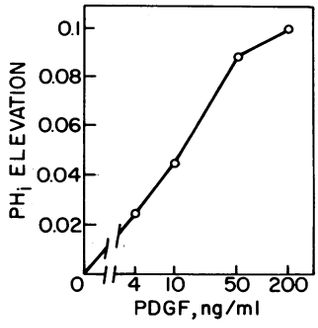

FIG. 5. Dependence of pH_i on PDGF concentration. Data represent the increase in pH_i 15 min after addition of PDGF, at which time pH_i has stabilized at a more alkaline value.

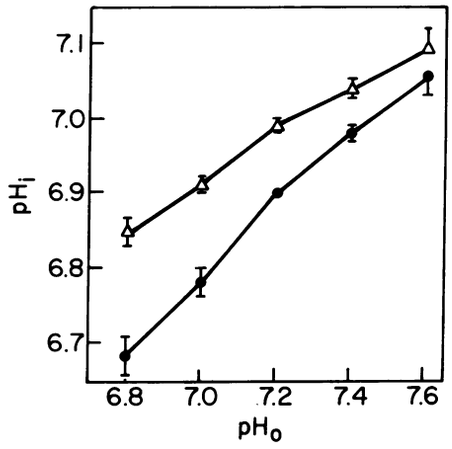

FIG. 6. Effect of extracellular pH (pH$_o$) on pH$_i$ in the presence and absence of PDGF. Fluorescence was initially recorded at pH$_o$ = 7.18. Cells were then switched to solution A at the indicated pH$_o$ values and incubated until no further change in fluorescence was observed. PDGF was then added and the increase in fluorescence was determined. pH$_i$ values are calculated relative to pH$_i$ = 6.90 at the initial pH$_o$ of 7.18 by using the calibration curve shown in Fig. 4. Results represent mean ± SD of four separate experiments.

pH$_i$ is complicated by the capability of the drug to quench the fluorescence of both fluorescein and 4',5'-dimethylfluorescein (50% quenching of DMFD fluorescence at 0.7 mM amiloride in free solution). Quenching is not due to spectral overlap and appears to depend on molecular interaction between amiloride and the fluorophore. Thus, any quenching in DMFD-loaded cells will depend on uptake of amiloride by the cells. As shown in Fig. 7 (trace A), addition of 3 mM amiloride causes a slow decline in the fluorescence emitted by the cells. This may reflect quenching due to amiloride uptake as well as inhibition of basal Na^+/H^+ exchange. On removal of amiloride, fluorescence increases rapidly, slightly "overshoots," and then returns to the initial value. Amiloride strongly inhibits the rate of alkalinization produced by PDGF and serum (Fig. 7, traces B and C). In fact, in the presence of amiloride, both PDGF and fetal calf serum initially promote cytoplasmic acidification; note that this acidification does not show a lag period. Removal of the drug results in a large overshoot of pH$_i$ that later declines to a level comparable with that observed in cells directly treated with PDGF or serum in the absence of amiloride.

Effect of External Na^+ and Ca^{2+} on PDGF-Dependent pH$_i$ Elevation. Incubation of cells in Ca^{2+}-free medium containing 0.5 mM EGTA has no effect on pH$_i$. Subsequent addition of PDGF results in a pH$_i$ elevation similar to that observed in the presence of 1.8 mM Ca^{2+} (data not shown). These results are in agreement with previous findings by Villereal (16) and by Rothenberg *et al.* (7) that external Ca^{2+} is not required for growth factor-stimulated $^{22}Na^+$ uptake.

Reducing the Na_o^+ concentration to 0.1 mM (Fig. 8, trace A) initiates a slow decline of pH$_i$. Subsequent addition of 130 mM Na^+ leads to a rapid increase in pH$_i$ that slightly overshoots and then returns to approximately the resting level. Addition of PDGF at 0.1 mM Na_o^+ (Fig. 8, trace B) initially facilitates the rate of decrease in pH$_i$. This could be due to stimulation by PDGF of Na^+/H^+ exchange at higher internal than external Na^+ concentration. However the rate of PDGF-stimulated acidification at low Na^+ concentration is only slightly reduced in the presence of amiloride, suggesting that the acidification is not mediated through Na^+/H^+ exchange (Fig. 8, trace C). Note also that in the absence of PDGF, amiloride does not significantly affect the rate of decrease in fluorescence caused by low Na_o^+ concentration. Since amiloride effectively inhibits Na^+/H^+ exchange at low Na^+ concentrations (17–20), the acidification at low Na^+ in the absence of PDGF may also be unrelated to Na^+/H^+ exchange. Alternatively, the decrease in fluorescence on addition of amiloride may reflect quenching of DMFD fluorescence (Fig. 7). On readdition of 130 mM Na^+ to cells that have been incubated with PDGF at 0.1 mM Na^+ (Fig. 8, trace B), pH$_i$ increases rapidly, overshoots, and declines to the normal stimulated level of approximately 0.1 pH unit above the initial pH$_i$. In cells previously incubated in 0.1 mM Na^+ without PDGF, subsequent exposure to PDGF at 130 mM Na^+ does not result in a large overshoot of pH$_i$ (data not shown). Thus, the overshoot phenomenon on readdition of Na^+ depends on the presence of PDGF during the incubation period in low Na^+. Results very similar to those shown in Fig. 8 were obtained when serum was used instead of PDGF. Note the similarity of the effects observed on inhibition of Na^+/H^+ exchange by either amiloride (Fig. 7) or by low external Na^+ (Fig. 8). In each case, PDGF stimulates an initial acidification. On readdition of Na^+ or removal of amiloride, there is a large overshoot, suggesting maximal activation of the Na^+/H^+ ex-

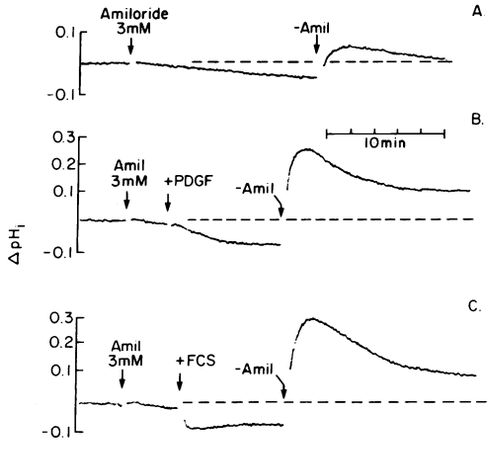

FIG. 7. Effect of amiloride on elevation of pH$_i$ by PDGF and serum.

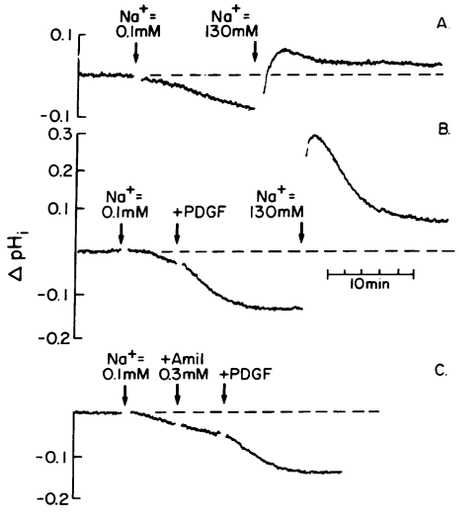

FIG. 8. Effect of PDGF on pH$_i$ at reduced Na^+ concentration. For Na^+-free solution, NaCl was replaced with tetramethylammonium chloride. Similar results were obtained using *N*-methyl-D-glucamine to replace NaCl. The estimated Na^+ concentration of 0.1 mM in this medium is due to the presence of Na^+ in the human serum albumin preparation.

change system by PDGF under conditions in which it cannot function followed by return to the pH_i usually observed in 130 mM Na^+ after addition of PDGF.

DISCUSSION

This paper reports that both serum and a defined growth factor can promote a sustained cytoplasmic alkalinization by stimulating Na^+/H^+ exchange. Different patterns of pH_i elevation are observed with serum and PDGF. Addition of serum initially generates a large transient increase in pH_i whereas PDGF addition results in a rather monotonic rise to a final pH_i level. The transiency of the initial pH_i elevation by serum may be related to the strong decline in serum-dependent $^{22}Na^+$ uptake after prior incubation of cells with serum (Fig. 2). However, previous exposure to serum or PDGF does not completely inactivate $^{22}Na^+$ uptake, and PDGF-dependent $^{22}Na^+$ uptake declines by only about 30% after 40 min of prior incubation with the hormone.* On the other hand, PDGF-dependent pH_i elevation is essentially complete within 10 min. This suggests that at steady state the rate of H^+ efflux through the activated Na^+/H^+ exchange is balanced by an increased rate of H^+ generation within the cell. The factors that are involved in pH_i homeostasis in the presence and absence of growth factors remain to be defined.

An interesting feature of the elevation of pH_i by both serum and PDGF is that it follows a lag period of about 2 min, during which a slight acidification is frequently observed. A similar lag period is observed for the stimulation of $^{22}Na^+$ uptake by PDGF and to a much lesser extent for the serum-stimulated uptake. The lag period for pH_i elevation by growth factors does not seem to reflect the time required for growth factor–receptor interaction because (*i*) its duration is essentially independent of growth factor concentration and (*ii*) on inhibition of Na^+/H^+ exchange, PDGF and serum promote cytoplasmic acidification that does not show a lag period. In A431 cells, EGF stimulates $^{22}Na^+$ uptake very rapidly (ref. 7 and unpublished results). We have recently found (unpublished results) that EGF also promotes an increase in pH_i in A431 cells and that the increase in pH_i follows a lag period similar in duration to that found in NR6 cells. A lag period for the elevation of pH_i but not for the stimulation of $^{22}Na^+$ uptake as in A431 cells may reflect a combination of acidifying and alkalinizing effects of the growth factor that nearly match each other at early times. The unusual lag period for the stimulation of $^{22}Na^+$ uptake by PDGF suggests that the mechanism of stimulation of Na^+/H^+ exchange by PDGF may be different from that of other growth factors.

What is the relationship between the stimulation of Na^+/H^+ exchange by growth factors and mitogenicity? The magnitude of pH_i elevation observed by both ourselves and by Schuldiner and Rozengurt (6) is rather small (0.1–0.15 pH unit) and pH_i elevation by PDGF decreases with increasing extracellular pH (Fig. 6). For comparison, the elevation of pH_i associated with the activation of the unfertilized sea urchin egg is about 0.5 pH unit. It should be cautioned however that the conditions we use for pH_i determination (physiological salt solution) are not fully compatible with those required for cell growth and that we can only measure an average pH_i change in a cell population; the actual pH_i elevation may be larger than the average if only a fraction of the cells respond to growth factors under the experimental conditions. Furthermore, small changes in pH_i may cause large changes in the activity of certain enzymes such as phosphofructokinase (21). However there is some evidence in the literature suggesting that stimulation of Na^+/H^+ exchange may be necessary but insufficient for the stimulation of cellular proliferation. Although all growth factors examined to date can stimulate an amiloride-sensitive Na^+ uptake, stimulation of this reaction in the absence of significant growth stimulation has been reported for Lys-bradykinin in W138 cells (4). In A431 cells, EGF promotes cytoplasmic alkalinization (unpublished results) but is not a mitogen for these transformed cells, suggesting that stimulation of Na^+/H^+ exchange by EGF can take place in cells that are not in a quiescent state. Stimulation of Na^+/H^+ exchange by growth factors may primarily be related to pH_i homeostasis following metabolic activation of quiescent cells, but further studies are necessary to establish the role of Na^+/H^+ exchange in mitogen action.

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Note Added in Proof. The synthesis and spectral properties of DMFD are described in ref. 22.

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* We assume that the observed amiloride-sensitive ^{22}Na flux represents Na^+/H^+ exchange; however, Na^+/Na^+ exchange may represent a significant component of the observed rate of $^{22}Na^+$ uptake (18).