The Na+/H+ Exchanger Is Constitutively Activated in P19 Embryonal Carcinoma Cells, but Not in a Differentiated Derivative*

RESPONSIVENESS TO GROWTH FACTORS AND OTHER STIMULI*

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We have examined the functional properties and growth factor responsiveness of the plasma membrane Na+/H+ exchanger in pluripotent P19 embryonal carcinoma (EC) cells and in a differentiated mesodermal derivative (MES-1) by analyzing the recovery of cytoplasmic pH (pHi) from an acute acid load under bicarbonate-free conditions. In the absence of exogenous growth factors, the mean steady-state pHi of undifferentiated P19 EC cells (7.49 ± 0.03) is 0.55 unit higher than the value of differentiated MES-1 cells (6.94 ± 0.01). In both cell types, recovery of pHi from an NH4+-induced acid load follows an exponential time course and is entirely mediated by the amiloride-sensitive Na+/H+ exchanger in the plasma membrane. Kinetic analysis indicates that the higher steady-state pHi, in P19 EC cells is due to an alkaline shift in the pHi sensitivity of the Na+/H+ exchange rate, as compared to that in MES-1 cells.

The Na+/H+ exchanger of MES-1 cells is responsive to epidermal growth factor, platelet-derived growth factor, serum, phorbol esters, and diacetylcholine, as shown by a rapid amiloride-sensitive rise in pHi. The mitogen-induced alkalinization is attributable to an alteration in the pHi sensitivity of the exchanger. In contrast, the Na+/H+ exchanger of P19 EC cells fails to respond to any of these stimuli. Similarly, hypertonic medium rapidly activates the Na+/H+ exchanger in MES-1, but not in P19 EC cells.

We conclude that the Na+/H+ exchanger in undifferentiated P19 EC stem cells is maintained in a fully activated state which is unaffected by extracellular stimuli, as if signal pathways normally involved in growth factor action are constitutively operative.

The molecular mechanisms underlying embryonic growth and development are largely unknown, although it seems likely that polypeptide growth factors and their receptor-linked signal pathways have a major role in mammalian embryogenesis. A suitable approach to studying embryonic growth control is to use in vitro model systems such as murine EC1 cells, the undifferentiated stem cells of teratocarcinomas, which share many properties with the inner cell mass cells of the pre-implantation embryo. Some EC cells are able to differentiate, either in vivo or in vitro, into a variety of nontumorigenic cell types that derive from the three primitive germ layers.

Differentiation of EC cells is accompanied by drastic changes in growth properties, as has been demonstrated for the pluripotent mouse P19 EC cell line. Undifferentiated P19 EC cells exhibit a highly transformed phenotype and grow rapidly in the complete absence of exogenous polypeptide growth factors. Upon differentiation these cells lose their transformed properties and their ability to proliferate autonomously. The differentiated derivatives show anchor- dependent, contact-inhibited growth and are dependent on the supplementation of exogenous growth factors for sustained proliferation.

As a first step in elucidating such potential signal pathways, we have characterized and compared the properties of the plasma membrane Na+/H+ exchanger in both P19 EC cells and in a mesodermal derivative (MES-1) in bicarbonate-free media. It is generally accepted that the Na+/H+ exchanger not only has a major housekeeping role in pH regulation but also may function as a signal transducer in the action of growth factors in that it mediates a rapid and persistent rise in pH, in stimulated cells. There is increasing evidence that this mitogen-induced cytoplasmic alkalinization has a permissive effect on the initiation of protein and DNA synthesis, at least in fibroblastic cells maintained in bicarbonate-free media.

We report here that, while both P19 EC and MES-1 cells have a normally functioning Na+/H+ exchanger in terms of pH regulation, the exchanger of MES-1 cells is highly responsive to growth factors and other agonists, whereas the Na+/H+ exchanger in P19 EC cells is in a permanently activated state, resulting in a much higher steady-state pH value and in a complete lack of responsiveness to extracellular stimuli. Our results suggest that certain signal pathways normally utilized by mitogens to activate the Na+/H+ exchanger in responsive somatic cells are constitutively active in autonomously growing EC stem cells. This could represent a critical mechanism involved in early embryonic growth control.

EXPERIMENTAL PROCEDURES

Materials—BCECF acetoxymethylester was obtained from HSC Research Development Corp. (Toronto, Canada). EGF was from Collaborative Research (Lexington, MA). Human PDGF (>95% pure) was kindly provided by C.-H. Heldin (Ludwig Institute, Uppsala,
Sweden. Other agents were obtained from the following sources: dimethylamidole from Merck Sharp and Dohme; monensin from Lilly, nigericin, phorbol esters, and diacylglycerol from Sigma.

Cells—P19 EC and MES-1 cells were cultured as described previously (5, 6). on gelatinized flasks in a 1:1 mixture of DMEM and Ham’s F-12 medium containing 7.5% PCS and buffered with 44 mM NaHCO3 in a 7.5% CO2 atmosphere. For fluorescence measurements, cells were grown to near confluence on gelatinized glass coverslips (3 x 0.8 cm), and then maintained in serum-free DMEM (buffered to pH 7.4 using 20 mM HEPES) for periods of 6–24 h prior to experimentation.

Fluorometric pH Monitoring—P19 EC and MES-1 monolayers were loaded with the pH-sensitive indicator BCECF by uptake of its membrane-permeable ester (11–13). Impermeant dye is then synthesized in situ by the action of cytoplasmic esterase. BCECF loading was done by incubating the monolayers for about 30 min at 37 °C in HEPES buffered DMEM (pH 7.0) containing 5 μM BCECF acetoxymethyl ester. After washing, the BCECF-loaded cells were inserted into a thermostatted cuvette in a Perkin-Elmer LS-3 fluorescence spectrometer, and the pH-dependent emission was continuously recorded at 33 °C (excitation, 506 nm; emission, 532 nm; slits, 5 x 10 nm). Cells were incubated in HEPES-buffered saline (HBS), unless indicated otherwise. The composition of HBS was: 140 mM NaCl, 5 mM KCl, 1 mM CaCl2, 1 mM MgCl2, 10 mM glucose, 20 mM HEPES at the indicated pH values. Calibration of cytoplasmic dye fluorescence as a function of pH was done by the H+ equilibration method using the H+/K+ ionophore nigericin (5 μM), which sets [H+]/[K+] = [K+]g/[K+]i, as previously described (11–13). Test solutions (2.0 ml; prewarmed to 33 °C) were exchanged by a rapid perfusion procedure (13).

Electrophysiology—Cells were grown in 35-mm dishes (gelatinized) and electrophysiological methods identical with those described previously were used (14, 15). Cells were impaled under microscopic control with a single microelectrode (3 M KCl-filled; resistance, 30–40 Mohm). Only impalements that yielded stable membrane potentials (Vm) for at least 1 min were considered to be reliable. The cultures were incubated in HBS (pH 7.35) at 33 °C. For experiments in which Vm was measured as a function of [K+]i, total [NaCl] + [KCl] was kept at 145 mM.

RESULTS

Electrophysiological Observations—In an initial series of experiments we examined the ionic membrane properties of both cell types using conventional electrophysiological techniques. The mean membrane potential (Vm) of P19 EC cells in serum-free medium is estimated at -50.5 ± 1.2 mV (n = 20), while the Vm value of MES-1 cells is slightly more negative (-57.6 ± 1 mV, n = 16). Fig. 1 shows the dependence of membrane potential on the external K+ concentration for both cell types. The curves are drawn according to the simplified Goldman equation given as in Fig. 1. From these graphs the intracellular K+ concentration is estimated at 146 mM for P19 EC and 149 mM for MES-1 cells, i.e. the value of [K+]i, where Vm becomes 0 (by extrapolation).

pH Measurements with Intracellularly Trapped BCECF—Cytoplasmic pH of P19 EC and MES-1 cells was estimated by loading nearly confluent monolayers with the fluorescent dansyl BCECF and calibrating the cytoplasmic fluorescence intensity by means of the high [K+]i-migrin method. [K+]i, in these experiments was chosen to be equal to the value of [K+]i, i.e 146–149 mM (see previous section).

At a constant pH of 7.35, the mean steady-state pH, of P19 EC cells, maintained in serum-free HEPES-buffered DMEM for at least 10 h, is estimated at 7.49 ± 0.03 (means ± S.E.; n = 7). By contrast, the steady-state pH, value of MES-1 cells under identical conditions is substantially more acidic (6.94 ± 0.01; n = 31). Since the mean membrane potential of both P19 EC and MES-1 cells is in the 50–60 mV range (interior negative), the steady-state pH, in both cell types is well above the electrochemical equilibrium value of ~6.4 predicted by the Nernst equation, which demonstrates the presence of an H+ -extruding mechanism that regulates pHi. Further insight: into the nature of this pHi-regulating system can be obtained by studying the recovery of pHi from a sudden cytoplasmic acidification (16, 17).

pH Recovery from Acidification Is Mediated by Na+/H+ Exchange—To examine the pHi-regulating mechanism(s) in both P19 EC and MES-1 cells, the cells were acid-loaded by the NH4+ prepulse method (12, 16, 17) under HCO3-/CO2-free conditions. Addition of 15 mM NH4Cl causes an immediate rise in pH of 0.4–0.6 unit, due to influx and subsequent protonation of the weak base NH3. The passive entry and dissociation of the less permeant weak acid NH2 follows, causing pH to decrease slowly. Removal of external NH4+ then evokes an acute cytoplasmic acidification, as NH3 leaves the cell immediately and an excess of protons is trapped intracellularly (Fig. 2). The rapid fall in pH is followed by a spontaneous, exponential recovery which is 90% complete within 4–5 min in both P19 EC (Fig. 2A) and MES-1 cells.
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A

B

C

D

E

F

Fig. 2. Kinetics of pHᵢ recovery from NH₄Cl-induced acid loads. Cells were loaded with BCECF and fluorescence intensity was recorded as described under “Experimental Procedures.” External pH was 7.35. A–C represent typical recordings from P19 EC cells and D–F from MES-1 cells. NH₄Cl concentration was 15 mM. In Na⁺-free media (−Na⁺) NaCl was replaced by choline Cl. Amiloride concentration, 1 mM. Note the initial showing of pHᵢ recovery in P19 EC cells (A) as discussed in the text and Footnote 2.

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It is seen that in P19 EC cells, there is an initial, rather slow phase of pHᵢ recovery, which lasts for about 30 s; this initial slowing is not observed in MES-1 cells (cf. Fig. 2, A and D). We have not investigated the mechanism(s) underlying this phenomenon, but we note that the initial portion of the pHᵢ recovery curve is unavoidably contaminated by the continuing efflux of NH₄⁺ from the cells, which tends to slow the recovery process. Since P19 EC cells probably have a smaller surface:volume ratio than MES-1 cells (6), it seems likely that complete washout of NH₄⁺ from P19 EC cells requires more time, which would explain the initial slowing of the pHᵢ recovery in these cells (Fig. 2A).

When external Na⁺ is replaced with choline, pHᵢ does not recover in either cell type (Fig. 2, B and E). Furthermore, pHᵢ recovery is rapidly and reversibly blocked by the Na⁺/H⁺ exchange inhibitor amiloride (1 mM) and its more potent analogue dimethylamiloride (50 μM) (Fig. 2, C and F).

From these results we conclude that pHᵢ recovery from cytoplasmic acidification in P19 EC and MES-1 cells, as in other vertebrate cells (16–18), is mediated by a Na⁺/H⁺ exchange system in the plasma membrane.

Intracellular Buffering Capacity—From the initial shifts in pHᵢ produced by exposing the cells to NH₄Cl, an estimate can be obtained of the intracellular buffering capacity (β), defined as the amount of OH⁻ that has to be added intracellularly to raise pHᵢ by 1 unit (1, 3). In 12 such experiments, the value of β was calculated to be ~10 mM/pH for P19 EC and 17 mM/pH for MES-1 cells, in reasonable agreement with previously measured values in mammalian cells (16, 17).

Kinetic Analysis of pHᵢ Recovery—To obtain further insight into the kinetic properties of the Na⁺/H⁺ exchanger in both cell types, we calculated the rate constants of exponential pHᵢ recoveries using an iterative curve fitting procedure to fit the pHᵢ time courses to the equation

\[ \frac{d(pHᵢ)}{dt} = k \cdot (pHᵢ(∞) - pHᵢ(0))e^{-kt} \]

where \( k \) is the rate constant, \( t \) is the time, and \( pHᵢ(∞) \) is pHᵢ at the new asymptotic steady state. Typical exponential curve fittings are illustrated in Fig. 3, A and B. The mean rate constants of exponential recovery were calculated to be 0.57 ± 0.02 min⁻¹ (mean ± S.E.; \( n = 4 \)) for P19 EC cells and 0.72 ± 0.04 min⁻¹ (\( n = 9 \)) for MES-1 cells. As expected from the marked differences in the resting pHᵢ of both cell types, the value of pHᵢ(∞) of P19 EC cells is ~0.5 unit more alkaline than that of MES-1 cells.

As in other systems (13, 16, 17, 19) and in agreement with the exponential time courses of pHᵢ recovery, the relationship between the rate of pHᵢ recovery, \( d(pHᵢ)/dt \), and the value of pHᵢ was found to be linear for both P19 EC and MES-1 cells (Fig. 3C). As is seen in Fig. 3C, the function d(pHᵢ)/dt versus pHᵢ for P19 EC cells is markedly shifted along the pHᵢ axis...
stimulate protein kinase C, such as phorbol esters and cell-permeable diacylglycerols, induce a rapid activation of the Na⁺/H⁺ exchanger in their target cells leading to an increase in the steady-state pH. We measured the effects of various growth factors and known kinase C activators on the pH of P19 EC and MES-1 cells using the fluorometric technique. As shown in Fig. 4, addition of FCS, EGF, phorbol esters, or diacylglycerols to MES-1 cells leads to a substantial cytoplasmic alkalinization, up to 0.3 pH unit above the initial steady-state pH. In most cases, there is a lag of 30–60 s, followed by a pH rise to a new steady state within 8–10 min. A similar pH response was observed with PDGF (25 ng/ml), although a small initial acidification was consistently observed (not shown). Table I (second column) summarizes the mean increases in pH for MES-1 cells in response to various stimuli.

The induced alkalinizations are not observed in Na⁺-free media (see Fig. 5 for a typical example with EGF and TPA) and the pH shifts are completely inhibited by 1 mM amiloride (data not shown). Fig. 5 illustrates how stimulation of the cells in the absence of Na⁺ followed by a shift to normal medium results in alkalinization without a detectable lag, suggesting that the events that take place during the lag period are independent of Na⁺ influx into the cells. From these results we conclude that the increases in steady-state pH are mediated by the Na⁺/H⁺ exchanger, as in other stimulated cells.

P19 EC Cells Fail to Raise Their Resting pH, in Response to Extracellular Stimuli. In marked contrast to MES-1 cells, the P19 EC cell line fails to increase its pH above the resting value when treated with growth factors or with phorbol esters and diacylglycerols (Table I). The lack of effect of EGF on pH probably has a trivial explanation, since P19 EC cells have very few EGF receptors (~900 receptors per cell; Ref. 6). However, the number of specific phorbol ester binding sites in P19 EC cells is relatively high and comparable with that of MES-1 cells and other differentiated derivatives of P19 EC cells (20). Yet, TPA and diacylglycerol are completely ineffective in raising pH, in P19 EC cells, in contrast to the findings with these agents in MES-1 cells (Table I).

In many cells, the Na⁺/H⁺ exchanger can also be activated by exposing the cells to hypertonic medium (21–23). This osmotic activation of the Na⁺/H⁺ exchanger appears to play an important role in volume regulation and occurs via an as yet unidentified pathway, apparently not involving protein kinase C (22). In view of the finding that the Na⁺/H⁺ exchanger of P19 EC cells remains unresponsive to activators of kinase C, we undertook pH measurements to test whether the exchanger of these cells can be stimulated by hypertonic media. For comparison, pH was also measured in osmotically activated MES-1 cells.

Increasing the osmolarity of the medium from 300 to 450 mosM by addition of sucrose or KCl results in a rapid activation of the Na⁺/H⁺ exchanger in MES-1 cells, as indicated by an amiloride-sensitive rise in pH, of ~0.25 unit; again, no pH shift is observed in P19 EC cells, even when the osmolality of the medium is increased by 200 mosM (Table I). Yet, the inability of P19 EC cells to raise their pH in response to any extracellular stimulus is apparently not due to a limited driving force for the Na⁺/H⁺ exchanger (given by the magnitude of the transmembrane Na⁺ and H⁺ gradients), since the steady-state pH in P19 EC cells can readily be increased by addition of the Na⁺/H⁺ ionophore monensin (5 μM; Table I).

Effects of Lowering Extracellular pH. Considering the relatively high steady-state pH value of P19 EC cells and in view of the lack of response to external stimuli, it is conceiv-
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**FIG. 4.** Time course of pHᵢ shifts in MES-1 cells induced by various stimuli. Nearly confluent MES-1 cells were maintained in serum-free DMEM for at least 10 h and loaded with BCECF as described under "Experimental Procedures." Agents were added where indicated (FCS, 5%, v/v; EGF, 100 ng/ml; TPA, 100 ng/ml; 1,2-oleoylacylglycerol (OAG) and dioctanoylglycerol (dC₈), 50 μg/ml; phorbol dibutyrate (PDBu), 250 ng/ml; 4α-phorbol di-dodecanate (4α-PDD), 10 μg/ml).

![Graph showing pHᵢ shifts](image)

**TABLE I**

Measurement of ΔpHᵢ by BCECF fluorescence

| Stimulus | ΔpHᵢ |
|----------|------|
| Dialyzed FCS, 5→v/v | 0.0 (3) | 0.32 ± 0.05 (4) |
| EGF, 100 ng/ml | 0.0 | 0.20 ± 0.02 (5) |
| PDGF, 25 ng/ml | ND* | 0.19 (2) |
| TPA, 100 ng/ml | 0.0 (10) | 0.22 ± 0.02 (7) |
| PdBu⁺, 250 ng/ml | ND | 0.15 (2) |
| 4α-PDD, 10 μg/ml | ND | 0.0 (2) |
| OAG, 50 μg/ml | 0.0 (2) | 0.13 ± 0.04 (4) |
| dC₈, 50 μg/ml | 0.0 (2) | 0.12 ± 0.02 (4) |
| Hypertonicity, 200 mosM⁺ | <0.03 (6) | 0.25 ± 0.04 (4) |
| Monensin, 5 μM | 0.41 ± 0.02 (4) | 0.44 ± 0.02 (4) |

* ND, not determined.

The abbreviations used are: PdBu⁺, phorbol dibutyrate; 4α-PDD, 4α-phorbol di-dodecanate; OAG, 1,2-oleoylacylglycerol; dC₈, dioctanoylglycerol.

* Induced by addition of either sucrose or KCl to the incubation medium.

**FIG. 5.** Time course of pHᵢ shifts in MES-1 cells induced by EGF and TPA under Na⁺-free conditions. Cells were incubated in Na⁺-free medium until pHᵢ stabilized at a new resting value. EGF (100 ng/ml) and TPA (100 ng/ml) were added where indicated. Na⁺-free medium was replaced by normal medium (140 mM Na⁺) by rapid perfusion.

![Graph showing pHᵢ shifts](image)
In this study we have examined and compared the properties of the Na⁺/H⁺ exchanger in mouse P19 embryonal carcinoma cells, being undifferentiated, phenotypically transformed pluripotent stem cells, and in the differentiated mesodermaI derivative MES-1 which has a nontransformed phenotype. While the rapidly proliferating P19 EC stem cells have no requirements for exogenous growth factors, the P19-derived MES-1 cells are growth factor-dependent showing a significant mitogenic response to serum, EGF, PDGF (6) and TPA.³ We, therefore, also compared the responsiveness of the Na⁺/H⁺ exchanger in both cell types to various extracellular stimuli.

The results of the present study demonstrate that the Na⁺/H⁺ exchanger is present in both cell types and functions normally in terms of pH; regulation, independent of the state of differentiation. However, the most important observation is that the exchanger in P19 EC cells cannot be activated by a variety of extracellular stimuli; by contrast, in MES-1 cells the exchanger is highly sensitive to growth factors, phorbol esters, and hypertonic medium as previously described for many somatic cells (reviewed in Refs. 7 and 24). Taken together, our observations strongly suggest that the exchanger in P19 EC cells is already in a fully activated state, as if the

³ C. L. Mummery, unpublished results.
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involvement of Na\textsuperscript{+}/H\textsuperscript{+} exchange activation are constitutively turned on. The following lines of evidence lend support to this notion: (a) the steady-state pH\textsubscript{i} in P19 EC cells is relatively high (~7.50) when compared to the value in MES-1 cells (~6.95); (b) pH\textsubscript{i} fails to decrease even after prolonged deprivation of exogenous growth factors; (c) the relationship between Na\textsuperscript{+}/H\textsuperscript{+} exchange activity (plotted as pH\textsubscript{i} recovery rate in Fig. 3) and pH\textsubscript{i} is shifted to more alkaline values, in a manner similar to that observed in MES-1 cells when activated by growth factors or phorbol esters (cf. Fig. 7C); that is, the pH sensitivity of the exchanger in P19 EC cells corresponds to that of activated MES-1 cells; (d) pH\textsubscript{i} cannot be raised further by any of several extracellular stimuli tested (serum, phorbol esters, hypertonicity, etc.); and (e) artificially lowering the resting pH\textsubscript{i} potentiates stimulus-induced alkalinization in MES-1 cells, but not so in P19 EC cells.

Our results further show that the MES-1 cell line is a convenient model system to investigate growth factor regulation of Na\textsuperscript{+}/H\textsuperscript{+} exchange. We and others have reported that extracellular stimuli activate the Na\textsuperscript{+}/H\textsuperscript{+} carrier through an apparent increase in the affinity for cytoplasmic H\textsuperscript{+} to a more alkaline pH, without changing the apparent Na\textsuperscript{+} affinity or V\textsubscript{max} (12, 19, 21, 25). In other studies of Na\textsuperscript{+}/H\textsuperscript{+} exchange in myoblasts (26), serum stimulates exchange activity by increasing both the internal H\textsuperscript{+} affinity and the V\textsubscript{max} for Na\textsuperscript{+}. Our present data on the kinetics of pH\textsubscript{i} recovery in MES-1 cells (Fig. 7), demonstrating both an alkaline shift in pH, dependence and an accelerated recovery at acidic pH\textsubscript{i}, suggest that it is likely that mitogens raise steady-state pH, not only by increasing the internal H\textsuperscript{+} affinity of the Na\textsuperscript{+}/H\textsuperscript{+} exchanger but also by enhancing its V\textsubscript{max}. Isotopic Na\textsuperscript{+} uptake studies should help to test this hypothesis.

A major question, to which we have no answers yet, is what mechanism underlies the apparent constitutive activation of the Na\textsuperscript{+}/H\textsuperscript{+} carrier in P19 EC cells. There is good evidence that protein kinase C can activate, either directly or indirectly, the Na\textsuperscript{+}/H\textsuperscript{+} exchanger in most cell types (27-29). It is therefore conceivable that kinase C is permanently activated in P19 EC cells, thus keeping the Na\textsuperscript{+}/H\textsuperscript{+} exchanger in its fully activated state. However, it has recently been shown that treatment of P19 EC cells with phorbol esters such as TPA drastically reduce kinase C activity in the soluble cell fraction, with a substantial increase in the particulate fraction when compared to untreated control cells in a manner very similar to that observed in TPA-stimulated MES-1 cells and many other cell types. Furthermore, TPA is an inducer of c-myec protooncogene expression\textsuperscript{4} and of morphological alterations in P19 EC cells (20). Together, these results make it less likely that kinase C in P19 EC cells is constitutively activated, suggesting that a different pathway is responsible for the fully activated state of the Na\textsuperscript{+}/H\textsuperscript{+} exchanger in P19 EC cells. Interestingly, alternative modes of activation, not involving kinase C, appear to be involved in the action of EGF and hyperosmolarity on Na\textsuperscript{+}/H\textsuperscript{+} exchange in fibroblasts (30) and lymphocytes (31), respectively. The biochemical nature of these activation pathways remains to be determined.

It remains possible, of course, that the observed permanent activation of Na\textsuperscript{+}/H\textsuperscript{+} exchange in P19 EC stem cells is attributable to the endogenous production and secretion of growth factors acting via membrane receptors on the signal pathway(s) that lead(s) to Na\textsuperscript{+}/H\textsuperscript{+} exchange activation. Such autocrine mechanisms might be essential in early embryogenesis, where rapid proliferation of pluripotent cells is required. Whether the present results on P19 EC cells can be explained by the "autocrine secretion" hypothesis is yet to be established.

Finally, it is noteworthy that constitutive activation of Na\textsuperscript{+}/H\textsuperscript{+} exchange is not a typical characteristic of autonomously growing tumor cells in general: the exchanger in epidermoid carcinoma cells (32), HeLa cells, and neuroblastoma cells (27), Ehrlich cells (33), and virally transformed fibroblasts \textsuperscript{5} can be activated to some extent by various agonists. Future studies should reveal whether a permanently activated Na\textsuperscript{+}/H\textsuperscript{+} exchanger and an elevated resting pH\textsubscript{i} are hallmarks of pluripotent embryonal carcinoma and embryonic stem cells in general. It seems plausible to assume that by keeping Na\textsuperscript{+}/H\textsuperscript{+} exchange permanently activated, cells maintain their steady-state pH\textsubscript{i} in a range permissive for protein and DNA synthesis (8, 34). Another challenge for further research is to investigate whether constitutive activation of the Na\textsuperscript{+}/H\textsuperscript{+} exchanger and, hence, an alkaline resting pH\textsubscript{i} has a regulatory role in the growth and differentiation characteristics in germ cell tumors and early embryogenesis.

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