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Pharmacologically different Na/H antiporters on the apical and basolateral surfaces of cultured porcine kidney cells (LLC-PK_1)

(ethylisopropylamiloride/transport activities/differentiation)

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ABSTRACT **Proximal tubule cells of the kidney contain, on their apical surface, an amiloride-sensitive Na/H antiporter that functions in Na reabsorption and proton secretion. We have investigated the localization of the antiporter in a cloned cell line of porcine renal origin, LLC-PK_1/Cl_4, which is often considered to be a useful model of the proximal tubule. Transport measurements were performed with differentiated monolayers grown on Nuclepore filters, permitting independent access to the apical and basolateral cell surfaces. In control experiments with LLC-PK_1/Cl_4 monolayers, three marker transport systems showed the expected polarity: 87% of ouabain-sensitive Rb uptake was at the basolateral surface, and 99% of Na-dependent α-methylglucoside transport and 93% of Na-dependent D-aspartate (L-glutamate) transport were at the apical surface. By contrast, the monolayers displayed significant Na/H antiporter activity (assayed as ethylisopropylamiloride-sensitive ^{22}Na uptake) at both cell surfaces, with an apical uptake rate amounting to 44% and a basolateral rate amounting to 56% of the total. Significantly, the apical and basolateral antiporters could readily be distinguished from one another on the basis of ethylisopropylamiloride sensitivity. The apical system had an IC_{50} of 13 μM, close to that reported for kidney brush border vesicle preparations, whereas the basolateral system had an IC_{50} of 44 nM, similar to values seen in undifferentiated LLC-PK_1 cells and other cultured cell lines. The PKE20 mutant, previously selected from LLC-PK_1/Cl_4 on the basis of resistance to ethylisopropylamiloride, was found to overexpress the more resistant antiporter both during rapid growth and on its apical cell surface at confluence; normal amounts of the more sensitive antiporter were seen on the basolateral surface of confluent PKE20 cells. Taken together, these results suggest that there are two distinct forms of the Na/H antiporter, which are under separate genetic control.**

The amiloride-sensitive Na/H antiporter, which carries out an electroneutral exchange of Na^+ for protons across the plasma membrane, is widely distributed in animal cells and tissues. The antiporter participates in the regulation of intracellular pH and cell volume and in the response of cells to mitogenic stimulus under defined conditions (reviewed in refs. 1–3). In the proximal tubule of the mammalian kidney, the Na/H antiporter is restricted to the apical cell surface (4), where it plays a major role in transcellular Na transport and urinary acidification. As a result, brush border membrane vesicles from the kidney are rich in antiporter activity and have been used to characterize the kinetic behavior of the system in great detail (2).

In recent years, established cell lines derived from the kidney have provided a useful alternative way to study the distribution, properties, and regulation of renal transporters. One such cell line is LLC-PK_1, which was isolated from pig kidney and is capable of differentiating at confluence into a polarized monolayer with many characteristics of the proximal tubule (5). Amiloride-sensitive Na/H antiporter activity has been observed in these cells both during rapid growth (6) and at confluence (7). However, there has not been a clear consensus as to the localization of the antiporter in LLC-PK_1 cells. Cantiello *et al.* (8) measured ^{22}Na uptake into confluent cells and reported, consistent with the situation in the proximal tubule, that all of the amiloride-sensitive uptake took place from the apical side. Subsequently, Montrose *et al.* (9) used the fluorescent pH indicator 2′,7′-bis(2-carboxyethyl)-5,6-carboxyfluorescein to follow the recovery of intracellular pH from an acid load. In the absence of bicarbonate, recovery required extracellular Na and was blocked by amiloride, indicating that it was indeed mediated by the Na/H antiporter. Surprisingly, however, in 22 of the 27 cells examined, the antiporter activity was found to reside on the basolateral surface, while only one cell conclusively displayed apical activity.

We have isolated (10) a mutant of LLC-PK_1 cells with a 3- to 5-fold increase in antiporter activity and a 100-fold decrease in sensitivity to ethylisopropylamiloride (EIPA). In the present study, we have grown the parent cells (LLC-PK_1/Cl_4) and the mutant (PKE20) to confluence on Nuclepore filters and have measured EIPA-sensitive ^{22}Na uptake on both sides of the monolayers. We find evidence for two pharmacologically distinct antiporters: an EIPA-sensitive form present on the basolateral surface and a more resistant form present on the apical surface. It is the latter form of the antiporter that exhibits increased activity in the PKE20 mutant. A preliminary report of this study has been published (11).

METHODS

Cells. LLC-PK_1/Cl_4 (a cloned, single-cell isolate of LLC-PK_1, hereafter referred to as Cl_4) was a gift from John Cook (Oak Ridge, TN). PKE20 was selected from Cl_4 based on its ability to survive acid-loading in the presence of EIPA, as reported elsewhere (10). The original uncloned LLC-PK_1 cell line was obtained from the American Type Culture Collection (CRL 1392). Stock cultures were maintained as described (12).

Uptake Studies on Filters. For uptake studies, cells were seeded in six-well Transwell filter dishes (Costar, Cambridge, MA) at a density of 1–3 × 10^5 cells per well in α minimal essential medium under 5% CO_2/95% air and were refed with

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Abbreviations: EIPA, ethylisopropylamiloride; EBSS, Earle's balanced salt solution.

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fresh medium on day 4 and every other day subsequently. Monolayers were routinely used on day 7, but similar results were obtained with cells grown as long as 14 days. For uptake measurements, the growth medium was aspirated, and both sides of the monolayer were rinsed with Hank's balanced salt solution buffered with 20 mM Hepes to pH 7.3. Uptake medium, consisting of suitably modified Earle's balanced salt solution (EBSS) buffered with 20 mM Hepes to pH 7.3 (see below), was then added together with radioactive substrate on one or the other side of the monolayer. After assay, the filters were rinsed three times in ice-cold 0.1 M $MgCl_2$ and solubilized in 0.5% $NaDodSO_4$. Incorporated radioactivity was measured by scintillation counting. Protein was measured by the method of Lowry *et al.* (13).

In the case of Na-dependent α-methyl glucoside uptake, the side of the monolayer to be assayed was treated with glucose-free EBSS containing 100 μM methyl (α-D-[U-^{14}C]gluco)pyranoside (methyl α-[^{14}C]glucoside) (0.2 μCi/ml; 1 Ci = 37 GBq) with or without Na; the opposite side was treated with Na-free, glucose-free EBSS. For the measurement of Na-dependent D-aspartate uptake (via the L-glutamate carrier; ref. 14), the side to be assayed was treated with EBSS containing 100 μM D-[^{3}H]aspartate (1 μCi/ml) with or without Na; the opposite side was treated with Na-free EBSS. To determine ouabain-sensitive Rb uptake (as a measure of the Na/K pump), the rinse solution was replaced on both sides of the monolayer with EBSS with or without 250 μM ouabain. After 5 min, Rb uptake was measured in EBSS containing ^{86}Rb (1–2 μCi/ml), again with or without ouabain.

For assaying Na/H antiporter activity (as EIPA-sensitive ^{22}Na uptake), the rinse solution was replaced on both sides of the filter with Na-free EBSS containing 50 mM NH_4Cl to acid-load the cells (6). After 20 min this solution was replaced on the side to be assayed with EBSS containing 15 mM ^{22}Na (1 μCi/ml) with or without EIPA. On the opposite side, the NH_4Cl-containing solution was replaced with Na-free EBSS.

The Na/H antiporter activity of confluent monolayers grown on plastic was determined as described for rapidly growing cells (12) with the addition of a 10-min incubation in 4°C rinse solution to remove trapped extracellular ^{22}Na.

Materials. ^{22}NaCl (in aqueous solution) and methyl α-[^{14}C]glucoside were obtained from Amersham, and ^{86}Rb (in ≈0.5 M HCl) and D-[^{3}H]aspartic acid were from New England Nuclear. Ethylisopropylamiloride was a gift from Edward J. Cragoe, Jr. (Merck Sharp & Dohme).

RESULTS

Impermeability of Confluent Cell Monolayers. It was essential for this study that LLC-PK_1 cells be able to grow to confluence on Nuclepore polycarbonate filters, forming a tight monolayer that would be relatively impermeable to small molecules. The ability of Cl_4 to do so is illustrated in Fig. 1. With a bare filter, the addition of a trace quantity of ^{22}Na to the upper compartment was followed by a substantial movement of radioactivity across the filter in 5 min and by complete equilibration in 30 min. By contrast, when a monolayer of Cl_4 cells had formed on the filter, less than 0.1% of the ^{22}Na crossed to the lower compartment in 5 min, and only about 5% in 60 min. Evidence that the impermeability resulted from the formation of tight junctions by the cells came from a control measurement in which the monolayer was incubated in Ca-free medium for 30 min prior to adding ^{22}Na (Fig. 1); the breakdown of tight junctions in the absence of Ca (15, 16) led to a substantial increase in the permeability of the monolayer. The experiment of Fig. 1 and (except where mentioned) all further experiments to be described in this paper were carried out with 7-day-old cells, although control measurements revealed that, starting with an inoculum of 1–

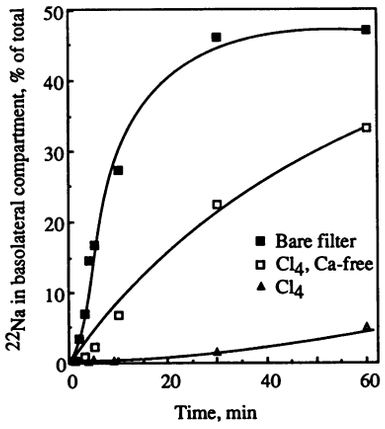

FIG. 1. Equilibration of Na across a monolayer of Cl_4 cells compared with that across a bare filter. In each case, the upper compartment of a well of a standard Transwell dish was filled with 2 ml of EBSS containing 15 mM ^{22}Na (1 μCi/ml), and the lower compartment was filled with 2 ml of Na-free EBSS. Aliquots were removed at intervals from the lower compartment for scintillation counting. All cells were 7-day-old monolayers. Ca-free cells were pretreated 30 min in medium containing 1 mM EGTA. Each point is the mean of two determinations from separate filters.

3×10^5 cells per well, a tight monolayer formed on or about the day 4 after seeding and was maintained for at least 14 days.

Localization of the Na/K Pump, the Na-Dependent Glucose Transporter, and the Na-Dependent L-Glutamate Transporter. The next step was to determine whether the confluent monolayers of Cl_4 cells displayed the expected polarity, with the apical cell surface facing the upper compartment and the basolateral surface facing the lower compartment. To examine this point, three well-studied transport systems were chosen as markers. One system, the Na/K pump, located on the basolateral surface of proximal tubule cells, has been detected previously on the basolateral surface of LLC-PK_1 cells by measurements of ouabain binding (17). In the present case (Fig. 2 *Left*), 87% of the ouabain-sensitive ^{86}Rb uptake by Cl_4 monolayers occurred from the lower compartment of the filter apparatus, confirming that the cell surface facing the filter is the basolateral surface.

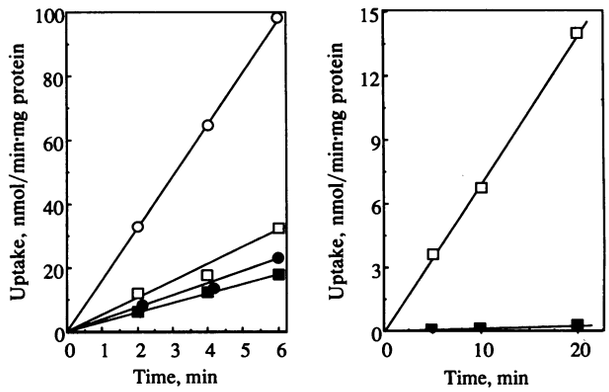

FIG. 2. Activity of apical and basolateral transporters in Cl_4 monolayers. (*Left*) Apical (□, ■) and basolateral (○, ●) ^{86}Rb uptake in the absence (□, ○) and presence (■, ●) of 250 μM ouabain. The initial rates of ouabain-sensitive uptake were 2.2 nmol/min·mg of protein (apical) and 12.4 nmol/min·mg of protein (basolateral). (*Right*) Apical methyl α-[^{14}C]glucoside uptake in the presence (□) and absence (■) of Na. All basolateral values fell within the Na-free apical symbols. The initial rates of Na-dependent uptake were 700 pmol/min·mg of protein (apical) and 10 pmol/min·mg of protein (basolateral). Each point is the mean of two determinations.

The Na-dependent glucose transporter serves as an appropriate marker for the apical surface based on previously published results with proximal tubule cells (18) and LLC-PK_1 cells (19, 20). The Na-dependent L-glutamate transporter is also apically located in LLC-PK_1 cells (14), although distributed on both surfaces in the proximal tubule (21). Once again, the results of the present experiments were consistent with expectations, since 99% of methyl α-[^{14}C]glucoside uptake (Fig. 2 *Right*) and 93% of D-[^{3}H]aspartate uptake (not shown) by Cl_4 monolayers occurred from the upper compartment of the filter assembly.

Localization of Na/H Antiporter Activity. In our previous work with rapidly growing Cl_4 cells (6, 12) acid loading was required to stimulate measurable amiloride-sensitive ^{22}Na uptake; this requirement almost certainly reflects the fact that the Na/H antiporter has an internal regulatory site that must be protonated for the system to be active (22). Therefore, it was not surprising that Cl_4 monolayers, when assayed under basal conditions in the present study, displayed little EIPA-sensitive ^{22}Na uptake: only 3.7 nmol/min·mg of protein at the apical surface and 4.7 nmol/min·mg of protein at the basolateral surface. By contrast, when the cells were acid-loaded via a 20-min pretreatment with 50 mM NH_4Cl followed by transfer to a NH_4Cl-free medium containing ^{22}Na, the EIPA-sensitive uptake rate rose to 27.4 ± 3.9 nmol/min·mg of protein at the apical surface and 35.2 ± 4.8 nmol/min·mg of protein at the basolateral surface (mean ± SD for four experiments; Fig. 3 *Left* and Table 1). Neither value changed appreciably when the cells were grown for up to 14 days on the filter. Thus, differentiated monolayers of Cl_4 cells express significant antiporter activity on both cell surfaces, unlike the situation in the proximal tubule.

To ask whether there was any detectable difference between the antiporters on the two sides of the cell, both were titrated as a function of EIPA concentration. The apical and basolateral titration curves differed strikingly (Fig. 4 *Left* and Table 1): the basolateral IC_{50} value of 44 nM resembled that found in rapidly growing Cl_4 cells (20–31 nM; ref. 6 and below), whereas the apical IC_{50} value of 13 μM was 300-fold higher, closer to values reported for brush border membrane vesicles of mammalian kidney (4.7 μM in rabbit; 7.1 μM in rat; ref. 23).

If LLC-PK_1 cells truly develop a new relatively drug-resistant form of the Na/H antiporter on their apical surface at confluence, and if this differentiated property is relatively independent of the substratum on which the cells are grown, then one might expect to see a shift in the IC_{50} on plastic dishes when cells progress from the rapidly growing state to a confluent monolayer, where only the upwardly facing apical surface is readily accessible to the assay medium. Such a shift does occur (Table 2). The IC_{50} in rapidly growing Cl_4 cells was measured to be 31 nM EIPA and rose to 3.6 μM in confluent cells.

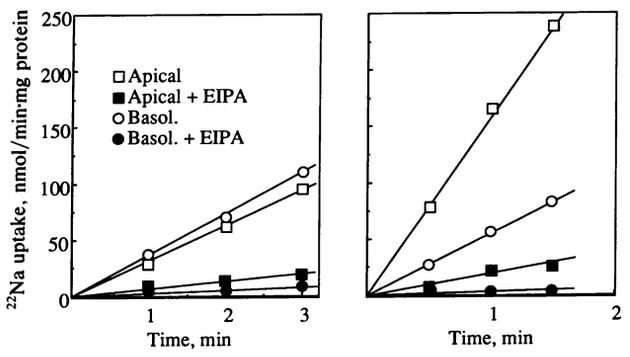

FIG. 3. Apical and basolateral activity of the Na/H antiporter in Cl_4 (*Left*) and PKE20 (*Right*) monolayers. ^{22}Na uptake was measured in the presence and absence of 100 μM EIPA. In this experiment the EIPA-sensitive rates were: for Cl_4, 25.4 nmol/min·mg of protein (apical) and 36.9 nmol/min·mg of protein (basolateral); for PKE20, 142.6 nmol/min·mg of protein (apical) and 53.4 nmol/min·mg of protein (basolateral). Note the different time scale on the ordinates. Each point is the mean of two determinations.

Table 1. Antiporter activity in parent and mutant cells grown on filters

Cell line	Apical Rate*	Apical IC_{50},† μM	Basolateral Rate*	Basolateral IC_{50},† nM
Cl_4	27.4 ± 3.9	13	35.2 ± 4.8	44
PKE20	153.2 ± 24.4	10	44.3 ± 10.2	29
LLC-PK_1	116.3‡	10	54.2‡	20

*EIPA-sensitive ^{22}Na uptake after acid stimulation (nmol/min·mg of protein; mean ± SD for four independent experiments).
†IC_{50} for EIPA.
‡Mean values from two independent experiments.

The IC_{50} for EIPA during rapid growth (2.6 μM) and that at confluence (3.9 μM) (Table 2).

Localization of Transporters in the PKE20 Mutant. The PKE20 mutant has been found (10) to display a 3- to 5-fold increase in Na/H antiporter activity when assayed during rapid growth on plastic dishes. The results for filter-grown monolayers are shown in Fig. 3 *Right* and Table 1. The ^{22}Na uptake rate of acid-stimulated PKE20 monolayers averaged 153.2 ± 24.4 nmol/min·mg of protein at the apical surface and 44.3 ± 10.2 nmol/min·mg of protein at the basolateral surface (mean ± SD for four experiments on 7-day-old cells). In control measurements, the three marker transport activities gave the expected polarities (92% of ouabain-sensitive ^{86}Rb uptake was basolateral; 98% of methyl α-[^{14}C]glucoside uptake and 95% of D-[^{3}H]aspartate uptake were apical), and data for the Na/H antiporter did not vary significantly when cells were grown for 14 (instead of the standard 7) days. Thus, the major change in antiporter activity of confluent PKE20 cells is a 5.5-fold increase at the apical surface.

When the two surfaces of filter-grown PKE20 monolayers were titrated with EIPA, the results were identical to those observed for the parent cells: the apical antiporter had an IC_{50} of 10 μM, while the basolateral antiporter had an IC_{50} of 29 nM (Fig. 4 *Right* and Table 1).

The antiporter activity of rapidly growing PKE20 cells has been found to be *ca.* 100-fold more resistant to EIPA inhibition than that of Cl_4 (10), suggesting that the mutant may express the apical form of the antiporter prematurely. Consistent with this idea, measurements on cells grown in plastic dishes revealed no significant difference between the

Localization of Transporters in Uncloned LLC-PK_1 Cells. The results for Cl_4 and PKE20 cells, while internally con-

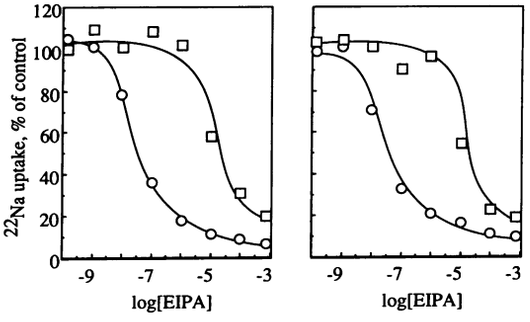

FIG. 4. Inhibition of apical (□) and basolateral (○) Na/H antiporters by EIPA in Cl_4 (*Left*) and PKE20 (*Right*) monolayers. The uptake of ^{22}Na was measured at 2 min in the presence of 0–750 μM EIPA. Each point is the mean of two determinations.

Table 2. Inhibition of Na/H exchange by EIPA in cells grown on plastic dishes

Cell line	IC_{50},* μM	
	Growing cells	Confluent cells
Cl_4	0.031 (7)	3.6 (3)
PKE20	2.6 (6)	3.9 (2)
LLC-PK_1	0.032 (2)	2.8 (1)

*Mean values are given from the indicated number of experiments in parentheses; IC_{50} for EIPA.

sistent with one another, do not correspond to either of the two previously mentioned studies with confluent LLC-PK_1 cells (8, 9). A critical aspect of the earlier studies, and one which in principle might account for the observed variability, is that both were carried out with descendants of the original uncloned LLC-PK_1 cell line (24), which is known to be heterogeneous. To come as close as possible to those cells in the present study, the standard uncloned LLC-PK_1 line was obtained from the American Type Culture Collection and subjected to the kinds of transport measurements described above. Control experiments gave the expected results for marker distribution: 87% of Na/K pump activity was basolateral, while 99% of Na-dependent methyl α-glucoside uptake and 95% of Na-dependent D-aspartate uptake were apical. Once again, however, there was significant acid-stimulated EIPA-sensitive ^{22}Na uptake on both cell surfaces: 116.3 nmol/min·mg of protein at the apical surface and 54.2 nmol/min·mg of protein at the basolateral surface (Table 1). Although the actual rates of uptake were somewhat higher than those in Cl_4, the IC_{50} values for EIPA were similar, with an apical value of 10 μM and a basolateral value of 20 nM. On plastic dishes, the IC_{50} values for uncloned LLC-PK_1 cells were 32 nM during rapid growth and 2.8 μM at confluence (Table 2). Thus, under the conditions of our experiments, the uncloned LLC-PK_1 cells also appear to express two Na/H antiporters in the differentiated state but only a single (sensitive) antiporter during rapid growth. It must be stressed, however, that owing to the heterogeneity of the original LLC-PK_1 cell line, we cannot be certain that the cells used in the present experiments were identical to those used in the two studies referred to above.

DISCUSSION

The key finding of the present study is that confluent LLC-PK_1 cells possess two pharmacologically distinct forms of the Na/H antiporter, which appear to be under separate genetic control. In its IC_{50} for EIPA (*ca.* 20 nM), the basolateral form resembles that seen in rapidly growing LLC-PK_1 cells (6, 10) and other cultured cells (25–27) and may represent a "housekeeping" antiporter used for intracellular pH regulation in the undifferentiated state. The apical form, with an IC_{50} of *ca.* 10 μM, resembles that seen in kidney brush border membrane vesicles (23) and may represent an antiporter that is somehow specialized for the transepithelial transport of sodium and protons. Similarly, Knickelbein *et al.* (28) have described two different antiporters in membrane vesicles from rabbit ileum. One form, present in brush border (apical) vesicles, has an IC_{50} for amiloride of 90 μM; the other, in basolateral vesicles, has an IC_{50} for amiloride of 6.5 μM. Further work is needed to learn whether the two forms observed in these systems are post-translationally modified products of a single gene or are separate products of two different genes.

In the meantime, Fig. 5 depicts the simplest interpretation of the difference between Cl_4 and PKE20 cells. In the Cl_4 parent, the EIPA-sensitive antiporter is distributed over the plasma membrane during rapid growth but becomes restricted to the basolateral surface at confluence; simultaneously, the EIPA-resistant antiporter begins to be expressed and is directed to the apical surface. In the PKE20 mutant, selection has led to an early over-expression of the resistant antiporter. This form is properly segregated to the apical surface at confluence, exposing the sensitive form on the basolateral surface. (It is quite possible that a small amount of the sensitive antiporter is already present in rapidly growing PKE20 cells but is masked by the relative excess of the resistant form.) The testing of this model awaits the identification of the Na/H antiporter gene(s).

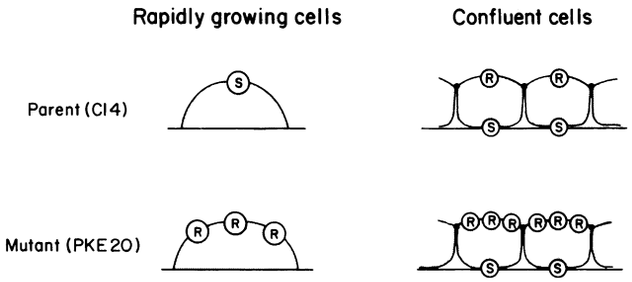

FIG. 5. Model for the distribution of sensitive (S) and resistant (R) forms of the Na/H antiporter in LLC-PK_1/Cl_4 and PKE20.

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1. Grinstein, S. & Rothstein, A. (1986) *J. Membr. Biol.* **90,** 1–12.
2. Mahnensmith, R. L. & Aronson, P. S. (1985) *Circ. Res.* **56,** 773–788.
3. Pouyssegur, J., Franchi, A., Kohno, M., L'Allemain, G. & Paris, S. (1986) *Curr. Top. Membr. Transp.* **26,** 202–218.
4. Ives, H. E., Yee, V. J. & Warnock, D. G. (1983) *J. Biol. Chem.* **258,** 13513–13516.
5. Gstraunthaler, G., Pfaller, W. & Kotanko, P. (1985) *Am. J. Physiol.* **248,** F536–F544.
6. Haggerty, J. G., Cragoe, E. J., Jr., Slayman, C. W. & Adelberg, E. A. (1985) *Biochem. Biophys. Res. Commun.* **127,** 759–767.
7. Moran, A. (1987) *Am. J. Physiol.* **252,** C63–C67.
8. Cantiello, H. F., Scott, J. A. & Rabito, C. A. (1985) *J. Biol. Chem.* **261,** 3252–3258.
9. Montrose, M. H., Freidrich, T. & Murer, H. (1987) *J. Membr. Biol.* **97,** 63–78.
10. Haggerty, J. G., Agarwal, N., Cragoe, E. J., Jr., Slayman, C. W. & Adelberg, E. A. (1988) *Am. J. Physiol.*, in press.
11. Agarwal, N., Haggerty, J. G., Reilly, R. F., Slayman, C. W. & Adelberg, E. A. (1987) *J. Cell Biol.* **105,** 331a (abstr.).
12. Agarwal, N., Haggerty, J. G., Adelberg, E. A. & Slayman, C. W. (1986) *Am. J. Physiol.* **251,** C825–C830.
13. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193,** 265–275.
14. Rabito, C. A. & Karish, M. V. (1983) *J. Biol. Chem.* **258,** 2543–2547.
15. Martinez-Palomo, A., Meza, I., Beaty, G. & Cereijido, M. (1980) *J. Cell Biol.* **87,** 736–745.
16. Gumbiner, B. & Simons, K. (1986) *J. Cell Biol.* **102,** 457–468.
17. Mills, J. W., MacKnight, A. D. C., Dayer, J.-M. & Ausiello, D. A. (1979) *Am. J. Physiol.* **236,** C157–C162.
18. Silverman, M. & Black, J. (1975) *Biochim. Biophys. Acta* **394,** 10–30.
19. Rabito, C. (1981) *Biochim. Biophys. Acta* **649,** 286–296.
20. Misfeldt, D. S. & Sanders, M. J. (1981) *J. Membr. Biol.* **59,** 13–18.
21. Sacktor, B., Rosenbloom, I. L., Liang, C. T. & Cheng, L. (1981) *J. Membr. Biol.* **60,** 63–71.
22. Aronson, P. S., Nee, J. & Suhm, M. A. (1982) *Nature (London)* **299,** 161–163.
23. Friedrich, T., Sablotni, J. & Burckhardt, G. (1987) *Biochem. Biophys. Res. Commun.* **144,** 869–875.

24. Hull, R. N., Cherry, W. R. & Weaver, G. W. (1976) *In Vitro* **12,** 670–677.
25. Zhuang, Y.-X., Cragoe, E. J., Jr., Shaikewitz, T., Glaser, L. & Cassel, D. (1984) *Biochemistry* **23,** 4481–4488.
26. L'Allemain, G., Franchi, A., Cragoe, E. J., Jr., & Pouyssegur, J. (1984) *J. Biol. Chem.* **259,** 4313–4319.
27. Vigne, P., Frelin, C., Cragoe, E. J., Jr., & Lazdunski, M. (1983) *Biochem. Biophys. Res. Commun.* **116,** 86–90.
28. Knickelbein, R. G., Aronson, P. S. & Dobbins, J. W. (1988) *FASEB J.* **2,** A941 (abstr.).